SYNTHETIC STUDIES IN BENZOPYRONE SERIES AND STUDY OF ANTHOXANTHIN GLYCOSIDES FROM INDIAN PLANTS

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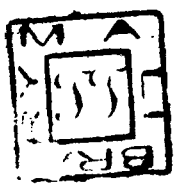

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MOHAMMAD ILYAS

I

R E S U M E

The first part of the thesis deals with the investigation of flavonoids from the bright yellow flowers of Argemone mexicana Linn. Ervatamia Coronaria Stapf. The flowers of Argemone mexicana have been found to contain a free aglycone and two new glycosides. The free aglycone m.p.304-306^{o} has been characterised as 3'-methoxy-4',5,7-trihydroxy flavonol (isorhamnetin) by its melting and mixed melting points, formation of its acetate m.p.203-204^{o}, Rf value and co-chromatography. The identity of the aglycone as isorhamnetin has been further evidenced by spectral studies (Ultra violet and infra red spectra). The glycoside m.p.165-67^{o} on acid hydrolysis gave an aglycone m.p.304-306^{o} and sugar. The aglycone has been identified as isorhamnetin as described earlier and the sugar as glucose by Rf value, co-chromatography and by the formation of osazone m.p.205-206^{o}. The glycoside gave an acetate m.p.135-36^{o} which on deacetylation furnished the original glycoside m.p.165-67^{o}. The methylation of glycoside followed by hydrolysis gave 3',4',5,7-tetramethyl quercetin m.p.192-94^{o} locating sugar residue at (C_3). The quantitative estimation of sugar by Somogyi's method showed the presence of 1 mole of sugar per mole of aglycone. The glycoside m.p.165-67^{o} is therefore characterised as isorhamnetin-3-glycoside.

The second glycoside m.p.208-10° gave on hydrolysis the same aglycone and the same sugar which were characterised as detailed earlier. The glycoside acetate m.p.148-50° regenerated the original glycoside m.p.208-10°. The methyl ether of the glycoside on hydrolysis gave a product m.p.284-85°. The partial methyl ether was identified as 3,3',4',5-tetramethyl-7-hydroxy quercetin which on acetylation gave 3,3',4'-5-tetramethyl-7-acetoxy quercetin m.p.174°. The formation of 7-hydroxy tetramethyl quercetin proved the attachment of sugar residue at (C_7). The quantitative estimation of sugar showed the presence of two moles of glucose per mole of aglycone. The glycoside m.p.208-10° is therefore characterised as isorhamnetin-7-diglucoside.

The white flowers of Ervatamia coronaria have been found to contain only one flavonoid component. The glycosidic nature of the flavonoid has been evidenced by the positive Molisch test obtained after hydrolysis and the chromatographic study of the sugars. The sugars were identified as glucose and rhamnose by Rf values using authentic checks. The aglycone m.p.276-78° was characterised as 4',5,7-trihydroxy flavonol (Kaempferol) by melting and mixed melting points and by the preparation of its acetate m.p.180-82°. The identity of the aglycone as Kaempferol was further confirmed by micro degradation. The components on chromatographic examination with authentic samples were characterised as phloroglucinol and p-hydroxybenzoic acid.

The glycoside gave an acetate m.p.158-60°. The deacetylation furnished the original glycoside m.p.222-24°, characterised as Kaempferol-3-rhamnose-glucoside.

The second part describes the syntheses of a new deoxybenzoin, (2,3-dihydroxydeoxybenzoin) and a new isoflavone (8-hydroxyisoflavone) The synthesis of 3,4-dihydroxydeoxybenzoin and o-methoxydeoxybenzoin in considerably improved yields has also been reported by a number of methods.

The condensation of pyrocatechol and phenyl acetic acid in presence of BF_3 gave 3,4-dihydroxydeoxybenzoin m.p.173-74° in 36% yield. By using veratrol in place of pyrocatechol 3,4-dimethoxydeoxybenzoin m.p.87-88° was obtained in 65% yield. The selenium dioxide oxidation of the methyl ether of deoxybenzoin gave a new α-diketone (3,4-dimethoxybenzil), m.p.114.5-115°.

The structure of the deoxybenzoin was supported by Beckmann rearrangement of its ketoxime. Two new compounds phenyl acet-3,4-dimethoxy anilide m.p.151-52° and N-benzyl (3,4-dimethoxy benzamide) m.p.134-36° were synthesised for comparison with the rearranged product. The product obtained by Beckmann rearrangement showed no depression in melting point on admixture with phenyl acet-3,4-dimethoxy anilide. The configuration of the ketoxim was, therefore, established as syn- benzyl type.

3,4-Dimethoxydeoxybenzoin has also been obtained by the interaction of benzyl magnesium chloride and 3,4-dimethoxybenzamide in 71% yield. The acylation of phenyl acetonitrile with ethyl

veratrate in presence of sodium ethoxide (Claisen acylation) followed by hydrolysis and decarboxylation gave 3,4-dimethoxydeoxybenzoin in 63% yield.

2,3-Dimethoxydeoxybenzoin b.p.170-73° (3 mm) has been synthesised by the interaction of benzyl magnesium chloride with 2,3-dimethoxybenzamide in 76% yield. The demethylation of the deoxybenzoin with a mixture of hydrobromic and acetic acids gave 2,3-dihydroxydeoxybenzoin m.p.79-81°. A number of carbonyl derivatives of 2,3-dihydroxydeoxybenzoin and its dimethyl ether have been obtained. The selenium dioxide oxidation of 2,3-dimethoxydeoxybenzoin gave 2,3-dimethoxybenzil, m.p.74° a new α-diketone. The structure of the hydroxydeoxybenzoin has been established by identification of phenylacetic acid and pyrocatechol as the products of alkaline degradation.

2,3-Dihydroxydeoxybenzoin on submitting to ethylformate-sodium synthesis formed 8-hydroxy isoflavone m.p.222-24°, acetate m.p.160°, methyl ether m.p.163°. The ethoxylation of the above deoxybenzoin gave 2-carbethoxy-8-hydroxyisoflavone m.p.213°, which on hydrolysis yielded 2-carboxy-8-hydroxyisoflavone m.p.262-63°. The decarboxylation of the acid isoflavone gave 8-hydroxyisoflavone m.p.222-24°. It showed no depression in melting point on admixture with the 8-hydroxy isoflavone obtained by ethyl-formate-sodium synthesis. The structure of the isoflavone has been proved by alkaline hydrolysis. The chromatographic examination of the product showed it to be 2,3-dihydroxy deoxybenzoin.

During the synthesis of 2,3-dimethoxydeoxybenzoin by Grignard method a side product m.p.53-54° in 12% yield was obtained. It was characterised as dibenzyl by comparison with an authentic sample obtained by the Clemensen's reduction of benzil. The above deoxybenzoin has also been obtained by the interaction of dibenzyl zinc with 2,3-dimethoxybenzoyl chloride (yield 34%).

o-Methoxydeoxybenzoin has been prepared by the use of Grignard reagent in 62% yield.

I

Acknowledgement

I wish to express my feelings of sincere appreciation and gratitude to Dr. Wasiur Rahman under whose guidance this work was carried out. Prof. M.O. Farooq was very helpful in providing facilities for the completion of my work and for this I am extremely grateful to him. I am also deeply indebted to Dr. A.A. Kidwai, Head of the, Department of Chemistry, who was kind enough to extend all possible help and to give many suggestions. Finally I acknowledge gratefully the ungrudging help given to me by my friends and colleagues from time to time.

C O N T E N T S

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T_H_E_O_R_E_T_I_C_A_L

THE GLYCOSIDES

The term glycosides embraces a large and remarkably varied group of organic compounds having the common property of furnishing saccharides or their oxidation products - the glycones - when hydrolysed by mineral acids or specific hydrolytic enzymes. Custom, however, restricts the term to those compounds which in addition to reducing sugars also yield one or more other substances which, not infrequently, are of an aromatic nature. The non-sugar constituent, is termed as an aglycone.

Laurent[1] (1852) was the first to collect together all substances which gave sugar on hydrolysis into a special group which he called glucoseamide and Berthelot[1] termed them later as saccharides. It is not known who originated the term glucoside. However, in the past, glucoside was the general name given to a class of organic substances which on hydrolysis gave sugar or a mixture of sugars. The term glucoside was based on the meagre knowledge of the group as no member was known which did not contain glucose as one of the products of hydrolysis.

The term glycoside is now officially used as general name for the group, irrespective of the sugar present, glucoside is the specific name used for those glycosides, the sugar constituent of which is glucose. In the past the glycosides were named ending in in, based on the plant in which they occur. It has been now proposed in France to substitute the suffix oside, to indicate the glycosidic nature. Thus asperulin becomes asperuloside. The non-sugar part of the glycoside is named as aglycone, a term originated by Japanese Chemists[2].

CLASSIFICATION OF GLYCOSIDES

The classification of glycosides is based upon the nature of aglycone. The aglycones include representatives of many of the numerous groups or hydroxyl compounds occuring in plants, ranging from small molecules such as ethyl alcohol, acetone, cyanhydrin to large ones such as the triterpenes, steroids (cardiac glycosides saponin etc.), hydroxyanthraquinones, anthocyanins and anthoxanthins. The following table (I) gives the classification representing one member of each group:-

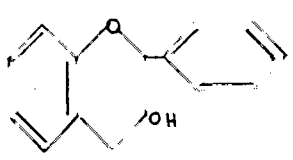
T A B L E - I

Class of Glycosides	Name of Glycosides	Hydrolysis products	Source	References
Alcoholic -	Gaultheriosido	Ethyl alcohol + glucose, xylose.	Gaultheria procumbens	3
Phenolic -	Goin	Eugenol + Vicianose	Geum urbanum	4,5,6,7
Cyanogenetic -	Vicianin	D-Mandelonitrile + vicianose	Wild vetch (seeds)	8,9
Thio -	Sinigrin	Allyl isothiocyanate + $KHSO_4$ + glucose	Black mustard	10
Anthocyanin -	Malvin chloride	Malvidin chloride + glucose (2 mol)	wild mallow	11,12
Coumarin -	Nodakon	Nodakenetin + glucose	Peucedanum	13,14,15
Saponin Triterpenic	Gypsophila	Gypsogenin + arabinose, rhamnose, glucose, galactose.	Gypsophila arrostii	16,17,18
Steroidal -	Dioscin	Diosgenin + D-glucose, L-rhamnose (2 mol)	Dioscorea nipponica	19
Sterol -	Gloriosol	Phytosterol + glucose	wheat germ & tubers of lily	20

Solanum alkaloid	Solanine	Solanidine + glucose, galactose, rhamnose.	potatoes	21,22,23
Cardiac -	Adonitoxin	Adonitoxigenin + rhamnose	Adonis vernalis	24,25
Anthraquinone -	Ruberthyric acid	Alazarin + xylose, glucose	Madder root	26,27
Nucleosidic -	Crotonoside	Isoguanine + D-ribose	Cotton bean	28,29
Phenylbenzopyrone				
Flavone -	Apiine	Apigenine + apiose, glucose	Parsley, celery	30,31,32
Flavonol -	Dactylin	Isorhamnetin + glucose (2 mol)	Grass pollen	33,34
Isoflavone -	Genistin	Genistein + glucose	Soya bean	35
Flavanone -	Hesperidin	Hesperitin (2 mol) + glucose (2 mol), rhamnose	Oranges and lemons	36,37,38,39
Chalkone -	Butein	Butin + glucose (2 mol)	Dhalia variabilis	40,41,42,43, 44

Phenyl Benzopyrone Glycosides:

Anthoxanthin glycosides: (Flavones, Isoflavones, Flavonols, Flavanones and Chalkones). Under the heading of flavone (word derived from the Latin for yellow) are included a number of yellow pigments occuring in the vegetative organs and in the petals of many plants. Owing to their close relationship to blue colouring matters known as anthocyanins, Willstater and Everest[45] proposed the adoption for them of the generic term, Anthoxanthin, first suggested by Marquart in 1835. The flavone nucleus occurs with variation in the oxidation levels of the C_3- portion of the molecule. The range of oxidation level extends from the highly reduced catechin type I to the highly oxidised flavonol XI.



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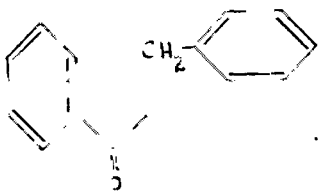

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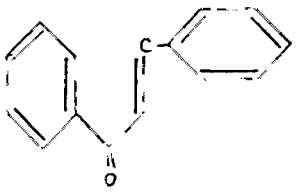

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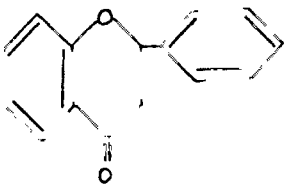

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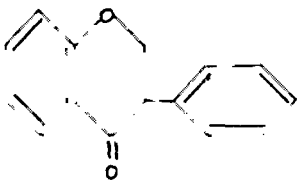

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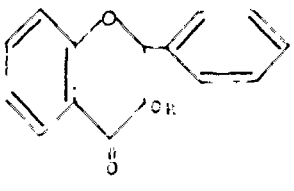

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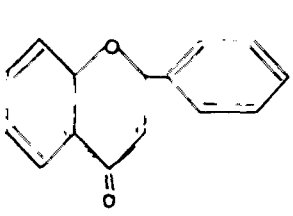

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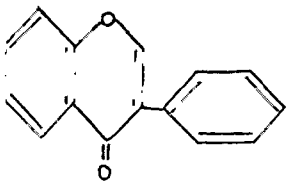

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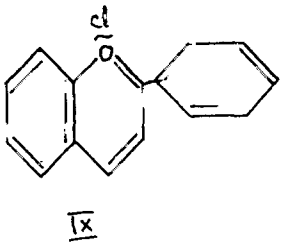

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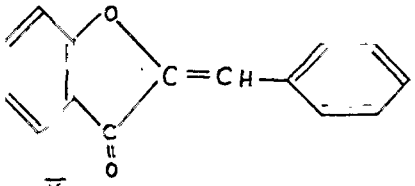

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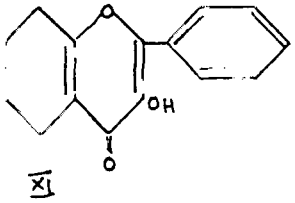

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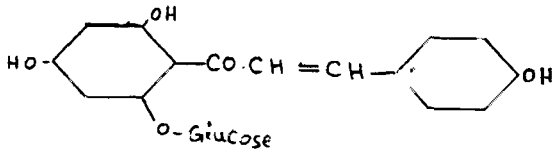
The following table II gives a brief out line:

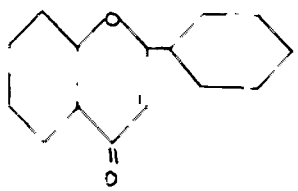
T A B L E - II

Figure	Compound Type	Oxidation State of C_3
I	Catechins	A - CH_2CHOH CHOH - B
II	Dihydro-chalcones	A - CO-CH_2-B(Iso:A-COCH-B) CH_3
III	Chalcones	A - CO-CH = CH - B
IV	Flavanones	A - CO-CH_2-CHOH - B
V	Isoflavanones	A -
VI	Flavanonols	A - CO-CHOH-CHOH - B
VII	Flavones	A - $COCH_2$ CO - B
VIII	Isoflavones	A -
IX	Anthocyanins	A - CH_2 COCO - B
X	Aurones	A - COCO CH_2 - B
XI	Flavonols	A - COCO CHOH - B

The great majority of the naturally-occurring flavonoid substances possess a phloroglucinol-derived ring A and catechol-derived ring B. No naturally occuring chalcone possessing the phloroglucinol-derived ring A with free hydroxyl group is known. Narasimhachari and Seshadri[46] have pointed out that when 5-hydroxyl group is present in the flavanone, the chalcone-flavanone isomerism is strongly on the side of the flavanone because of the resulting hydrogen bonding stabilization of the ring. Salipurporide XII is stable in the chalcone form, having a glucosidoxy residue in place of one of the ortho hydroxyl groups.



Variation in the structure of the A-ring extends from the simplest case of flavanone itself[47,48] VII to that in which hydroxyl groups are found in the 5,6,7 and 8-positions of the ring XIII.



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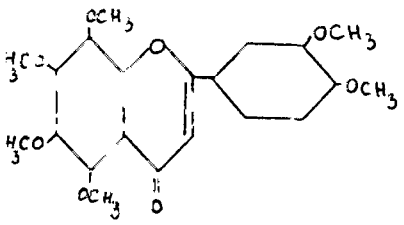
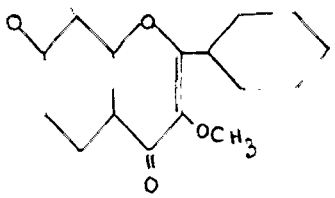


Variations in the hydroxylation pattern of the B-ring are relatively more limited. Compounds possessing the 4'-hydroxyl and the 3',4'-dihydroxyl groupings make up the bulk of the known compounds. Substances with no hydroxyl groups and with 2'-hydroxyl groups are known but rare. The 3',4',5'-trihydroxy B-ring occurs commonly but in a restricted group of compounds.

Aside from its presence in irigenin (3',5,7-trihydroxy-4'5',6-trimethoxy isoflavone) and robinetin, the occurence of the pyrogallol derived B-ring is most common in compounds having the 5,7,3',4',5'-hydroxylation pattern in the aromatic rings.

The alkylation of the hydroxyl groups of the flavonoid compounds, with the formation of methoxyl and methylenedioxy groups can give rise to numerous derivatives for each polyhydroxy compound. Modification of flavonoid hydroxyl groups by other than methylation or methylenation (of ortho-hydroxyl groups) can occur in natural polyphenols but is uncommon in the flavonoid compounds. Karanjin XIV possesses a furo-ring at the 7,8-positions.



XIV

The anthoxanthins in the form of their glycosides frequently are but faintly yellow in colour, the sugar free compounds generally having a deeper yellow colour than the glycosides. The sugar residue may be attached in any of most of the available positions in the molecules, but in certain classes of compounds glycosylation is restricted to certain positions. The anthocyanins bear sugar residue only in the 3-position if monoglycosides or biosides, and in the 3,5-positions if diglycoside. Flavonoid compounds bear the sugar residue in 3'-,4'-,3-,5-, 7- and 8-position. No flavone-6-glycoside is known and 4'-glycosides are very rare in nature. Flavonols occur most commonly as 3-glycosides; in flavones, 7-glycosides represent the largest class. The most commonly occuring sugars, that are found in combination with flavonoid aglycones, are D-glucose, L-rhamnose and D-galactose and biosides containing rutinose (β-L-rhamnosido-6-D-glucose). In addition to these, glucuronides, arabinosides, an apioside (apiin) is also known.

The Isolation and Identification of Flavonoid Compounds from Plant Materials.

During the past two decades many fundamental advances have been made in methods for the detection, separation, and structural determination of the water-soluble plant pigments. While relatively few fundamental changes have been introduced into the procedures for the large-scale isolation of flavonoid substances, the development of chromatographic and partition techniques, and the increasing application of absorption spectrometry have made available powerfull tools for the detailed examination of the complex mixtures of plant pigments.

Since plant tissues usually contain glycosidases as well as enzymes of other kind, autolytic processes may ensue subsequent to collection of the fresh material, resulting either in the hydrolysis of glycosides or the destruction of sensitive compounds. This may lead to the erroneous description of the plant constituents. Immediate and rapid drying of the plant material usually preserves it in a form substantially equivalent to the fresh material.

No general methods are available for the isolation of glycosides. The flavonoid compounds range in solubility from ether-soluble, water-insoluble, ether-alcohol-soluble,

ether-insoluble to water-soluble glycosides. Consequently no single extraction procedure is ideally suited to all plant materials. The first systematic scheme developed for the characterisation of one class of the water-soluble pigment was that elaborated by Robinson[49,50] for the rapid identification of the anthocyanins.

In general, the flavonoid compounds of fresh or desiccated plant materials can be completely extracted by means of ethyl or methyl alcohols, but it is often advantageous, especially when dried material is used, to carry out a systematic series of extractions with the use of three or four solvents of increasing polarity. A preliminary extraction of dried, powdered plant material with low boiling petroleum ether or carbon tetrachloride is effective in removing waxy materials. Petroleum ether-soluble flavonoids are of relatively infrequent occurrence, and such a pre-extraction usually removes non flavonoid constituents. However recently Lindstedt[51,52] has found in ether and petroleum extracts of heartwoods, hydroxylated flavonoid and anthrone pigments.

Since most flavonoid glycosides are rather readily hydrolysed by acids, care must be taken, especially when fresh material is used, to prevent the decomposition

of glycosides during extractions with boiling solvents. Rapid exposure of the plant to boiling alcohol is effective in inactivating hydrolytic enzymes but the materials in the extract are still exposed to the danger of hydrolysis by accompanying plant acids. It is customary to carry out long continued extractions in a soxhlet extractor with the addition of a small amount of calcium carbonate to the liquid in the boiler.

The ability of certain substances to form insoluble precipitates when treated with lead acetate, and the effect of pH upon precipitability, offers a useful mean: of separating or purifying many compounds.

In general, flavones, chalcones and aurones containing free ortho-hydroxyl groups in the B-ring, as in luteolin, quercitin, butein, aureusidin and leptosidin (and their A-ring glycosides) give deep yellow to red precipitates when their alcoholic solutions are treated with neutral lead acetate. After centrifugation and washing, the precipitate is suspended in alcohol and decomposed with a stream of hydrogen sulphide. After removal of the lead sulphide the regenerated substance is isolated from the alcoholic filtrate.

The filtrate from the original precipitation may be freed of lead with hydrogen sulphide, or basic lead acetate may be added to precipitate a second group of lead salts. These are decomposed and the products isolated as in the first instance.

Lead acetate is often effectively used to clarify extracts when no usable precipitate is actually formed. After the addition of the lead solution hydrogen sulfide is passed in and the precipitated lead sulfide, along with adsorbed colored and colloidal impurities, is removed. The clarified filtrate may then be processed in the usual ways. Lutokhin and Byvshikh[53] have recommended the use of zinc sulfate and potassium ferrocyanide as an alternative method for clarifying plant extracts. The precipitated zinc ferrocyanide forms a gel which carried down impurities. The use of this reagent with extracts containing flavonoid compounds has not been investigated with regard to the possible removal of flavonoid substances.

Uo, Fukushima and Kondo have found that katsuranin (3, 5,7,4'-tetrahydroxyflavanone) is not precipitated when lead acetate is added to the concentrated alcoholic extract of the wood of Cercidiphyllum japonicum, and is found in the filtrate from the lead precipitate.

Analysis by Chromatographic methods.

In recent years paper chromatography has been extensively used by various workers for the isolation and identification of flavonoids and their glycosides. Bate-Smith[54] was the first to give the description of the use of filter paper chromatography to the identification and separation of anthocyanidins, flavones and their glycosides. The flavonoid compounds have proved to be ideally suited to this elegant and powerful technique by reason of their wide range of solubility characteristics, the changes that are brought about in partition characteristics and consequently in Rf values by hydrolysis of glycosides, the characteristic colours of the substances themselves in visible or ultra-violet light and the colours produced by the application of appropriate reagents to the chromatograms.

The detection of Rf values for eleven flavonoid pigments in chloroform, ethyl acetate, phenol and butanol, acetic acid, the separation of mixtures containing four to six of these pigments, and the use of colour developing sprays to locate and identify the pigments zones has been given by Wender and Gage[55]. The characteristic colours in ordinary light and an intense flourscence in

U.V. light have been observed by treating the chromatogram with basic and neutral lead acetate, alcoholic $AlCl_3$, Na_2CO_3, and boric - citric acid reagent.

Two demensional paper chromatography has been used to separate and identify nine phenolic constituents of pine heart-wood extracts[56]. The solvent consists of a water saturated mixture of equal volumes of benzene and ligroin containing traces of methyl alcohol. Best results are obtained by using the above solvent mixture in one direction and chloroform:methanol:ligroin (2:1:7 + $5H_2O$) in the other (at right angles to the previous direction). Tetrazotized benzidine is used as spray reagent. A paper partition chromatographic investigation of 48 Pinus Species has been done[57]. Results are summarized of experiments with 41 flavone derivatives[58] by using butanol: acetic acid mixture; ethyl acetate-m-cresol and phenol-water as solvents.

The behaviour of 38 flavonoids in 11 solvents has been studied[59]. Colour developments has been given by 8 chromogenic sprays (alcoholic $AlCl_3$, $ThCl_4$, $FeCl_3$ each 1%, aqueous basic lead acetate, lead acetate, Na_2CO_3 each 1%, ammonical $AgNo_3$ and Benedict's solution) or spots have been located under U.V.light before spraying.

Simpson and Garden[60] have discussed the application of paper chromatography to the study of chelate system. It has been shown that the pyrone carbonyl group of flavones forms stronger hydrogen bonds with the 5-than with 3-hydroxyl group. The ability of the carbonyl group to form bonds with both hydroxyl groups simultaneously has been observed. A group of 30 flavones, selected to provide most of the possible combinations of hydroxyl and methoxyl substitution in the 3-,5-,7-and 4'-positions and a few simple phenols have been run on paper chromatogram. RM values have been determined with these solvents, under substantially the same conditions as those recommended by Bate-Smith and Westall[61]. Comparison of the RM values of a number of 4'- and 3'-hydroxy flavones shows[62] that a 4'-hydroxyl group stablises the carbonyl-3-hydroxyl more than the carbonyl-5-hydroxyl chelate system.

Recent applications of chromatographic methods to the study of plant extracts have shown the great complexity of the mixtures of closely related substances that may be encountered in the extract of a single tissue. Ice and Nerder[63] have identified four distinct glycosides of quercetin in the leaves of vaccinium myrtilles. Swain and Nordstrom[64] showed that the petals of a blue Dahlia (Dandy) contain 3 glycosides of apigenin, two of luteolin, two

anthocyanins and several other flavonoid glycosides not as yet completely identified.

Although the complex mixtures of phenolic glycosides present in plant extract may readily be separated by paper chromatography[65] the individual components can not usually be identified by Rf values and colour reactions alone[66] because there may be present a large number of glycosides related to a single aglycone[67]. Even direct measurement of U.V. absorption spectra on paper chromatograms[66] does not permit differentiation between glycosides related to one aglycone.

The use of packed columns for the separation and isolation of flavonoid compounds has not been exploited extensively. In general the difficulties in column chromatography of polyphenolic compounds lie in the limitations of the adsorbents used. No generally satisfactory material has been found that will give good separations of macro amounts of structurally diversified groups of compounds, and the separations of micro and semi-micro amounts of material on paper sheet chromatograms remains a more practical method for the isolation of milligram quantities than presently available procedures involving the use of columns.

Alumina, the most generally used adsorbent for the separation of organic compounds, is unsatisfactory for use with flavonoid compounds. Grassman[67], Grassman and Lang[68], Clark and Levy[69] found that pigments adsorbed on alumina were eluted with difficulty or not at all.

Bradfield, Penney and Wright[70], Ice and Wender[66] separated seven individual catechin derivatives from green tea leaves by partition chromatography on water-silica gel columns with wet ether as the mobile phase.

Pearl and Dickey[71,72] and Ice and Wender[63] have found that Magnesol (hydrated magnesium acid silicate) is a useful adsorbent for the separation of polyphenolic substances. Ice and Wender[63] have separated mixtures of quercetin and morin, quercetin, rutin and quercitrin, xanthorhamnin, rutin and quercetin, naringin and hesperidin, and naringin and apigenin-7-rutinoside on columns of Magnesol. The mixtures were applied to the column in anhydrous acetone-solution and development (with the collection of eluate fractions) was carried out with water saturated ethyl acetate. Nordstrom and Swain[64] have commented on their inability to achieve satisfactory separations of flowers petal extracts on columns.

Ion-exchange resins have found use in the preliminary purification of plant extracts containing flavonoid compounds, and in the separation of polyphenolic constituents[73,74] of extracts of peaches. Morris, Gage and Wender[75] employed amberlite IRC-50 cation exchange resin in separations of the phenolic constituents of a variety of plant materials.

It is usually necessary to supplement the separations carried out on columns by paper chromatographic procedures in order to establish the homogeneity, purity and identity of the fractions obtained from the columns. The combination of ion exchange resins, magnesol zinc silicate[76] columns and paper chromatography promises to be a useful one in the study of plant extracts.

C O N S T I T U T I O N

The problem arising in the elucidation of the structure of glycosides involves the following important steps.

i) Hydrolysis giving rise to glycone and aglycone components.

ii) Characterisation of glycone or carbohydrate moiety.

iii) (a) Paper chromatography of sugars

(b) Column chromatography of sugars

(c) Quantitative estimation of saccharides

(d) Study of the stereochemical nature of the glycosidic union.

iii) Characterisation of the aglycone:

(a) Colour reactions

(b) Comparison of the aglycone and its derivatives with synthetic standards by mixed melts and chromatographic methods

(d) Spectrophotometric methods; Ultraviolet and infra-red absorption spectra.

(d) Degradation

(e) Synthesis and isomerisation

Hydrolysis: All glycosides are hydrolysed by treating with dilute mineral acids, sulphuric and hydrochloric with the production of sugar and aglycone. They are hydrolysed at different rates, some glycosides, e.g. gynocardin, being extremely resistant to acid hydrolysis. The same reaction may be affected by the agency of enzymes whose action is, however, specific, the β-glycosides being hydrolysed by β-glycosidase (emulsin), while α-glucosides

are hydrolysed by α-glycosidase (maltase). It is important to note that both di- or tri-saccharides present in glycosides as carbohydrate moiety are converted into monosaccharides during hydrolysis. The following pentoses are known in glycosides: D-and L-arabinose, D-xylose, and D-ribose. The occurence of D-arabinose is rare in nature. Barbaloin, from Barbalos albes, sapindus saponin and albizzia saponin appear to be some of the only authenticated sources of D-arabinose among the plant glycosides. Apiin, a glycoside from parsley seeds contains the unusual pentose apiose, which has a branched chain.

The cardiac glycosides, on hydrolysis, yield in addition to the common sugars, D-glucose and L-rhamnose certain deoxy sugars which are found no where else in nature. Thus digitalis glycosides contain digitalose (3-methyl-6-deoxy D-galactose or 3-methyl-D-fucose) and digitoxose (2:6-dideoxy-D-allose or altrose).

Uronic acid is a rare constituent of glycosides[77]. Baicalein and Scutellarin, flavone glycosides, contain D-glucoronic acid. It is also present in the saponin aescin from horse chestnut seeds, in the saponin from the bark of quillaia saponaria, from mistletoe from sugar beet and randia dumaturum[78] etc.

Neutralisation of the hydrolysate:

At the conclusion of the hydrolysis it is necessary to neutralise the acid before concentrating the solution. The solution is always concentrated below 40°. Barium carbonate is customarily used to neutralise the solution while sulphuric acid has been used for hydrolysis. Barium carbonate should be pure and freshly precipitated and to avoid certain transformation it should be used in cold; sometime it is difficult to remove completely the barium sulphate formed. However, this can be done by shaking with an intimate mixture of both acid and base-binding resins[79]. The greatest disadvantage of barium carbonate is that it partially absorbs uronic acids as well as glucoronic acid lactones if present in the hydrolysate. The hydrolysis in such cases is brought about by mean of hydrochloric acid. The hydrolysate is concentrated in vacuum over KOH pellets till it is neutral to litmus paper.

It is advantageous to use columns of suitable resins such as Amberlite IR-100 and 4B or "Dawex 2"[80] for the removal of acidity from the hydrolysate.

Hydrochloric acid when used as hydrolytic agent is usually neutralised by pure silver carbonate followed (in absence of uronic, and glucoronic acids and their

lactones) by hydrogen sulphide to remove dissolved (colloidal) silver.

(ii) Characterisation of glycone or carbohydrate moiety:

In the last several years the use of chromatography for the separation of different compounds has sprung as an uncontestable technique and is widely used.

(a) Paper Chromatography.

The use of paper chromatography as a means of gaining rapid and specific information regarding the saccharide composition is well established. In addition, the paper chromatogram provides a means of carrying out quantitative analysis with confidence and accuracy. Previously, the analysis of a mixture of sugars was a task of great difficulty, depending largely on precipitation by specific reagents[81]. But it must be borne in mind that the characterisation of a saccharide solely by its chromatographic behaviour is not unequivocal. In general, the isolation and identification of crystalline compounds and the preparation of appropriate crystalline derivatives having characteristic physical and analytical properties should always be the analyst's ultimate aim.

Qualitative paper chromatography: The separation of monosaccharides was first described by Partridge[82,83,84]. The chromatograms were run with a number of mixtures of solvents. Partridge has published a fairly complete Rf value table[83].

Ascending as well as descending techniques by the mobile fluid phase are used. The circular technique developed[85,86,87,88] has also been utilized and the spraying reagents for revealing the sugar spots are to be numbered by the scores viz. analine phthalate, analine phosphate, para-anisidine hydrochloride, ammonical silver nitrate, p-anisidine phosphate[89] etc.

Spots of authentic sugars should always be included in runs along with the unknown sugars. The inclusion of such control 'spots' greatly minimises the chances of erroneous deductions arising from unforseen temperature changes etc. The selection of suitable spraying reagents also aids the certainty of identification.

Hough et al[90-91] have done considerable amount of work on this aspect of sugar chemistry and sufficiently detailed reviews on the subject have appeared[89,92,93].

(b) Column Chromatography.

By the above procedure, however, it is not possible to distinguish either between D- and L-steriOisomers of the sugar, or between sugars such as fructose, sorbose, and tagatose which show similar properties on the paper chromatogram. The final proof of their identity, therefore, still depends on their separation and on their identification by means of physical properties, in particular, their optical rotations.

The application of chromatography to the carbohydrate field was first described by Reich[94] who observed that a mixture of the p-phenylazobenzoates of glucose and fructose yielded two coloured bands when developed on a column of alumina or silica gel. Since this publication, the method of adsorption analysis has been extended by many other workers to the separation of simple mixtures of sugars and their derivatives[92].

Mc Neely, Binkley and Wolfrom[95] described methods for the separation of sugars and their acetyl derivatives on columns of commercial clays, such as "celite", Magnesol" etc.

Hough, Jones and Wadman[96,97] used powdered cellulose columns for the separation of monosaccharides. Hydrocellulose columns[98] are reported to have a higher resolving power and a greater capacity for methylated sugars than cellulose columns[99]. Besides cellulose columns which are used aburdantly, other columns such as those of charcoal are also used[100]. An entirely new type of chromatographic procedure is the separation of polyhydroxy compounds using strongly basic ion exchange resin and borate buffers of various pH-values[101,102]. The use of ultra-violet and Infra-red absorption spectra in structural detection of sugar is also reported[103,104,105].

(c) Quantitative Determination of Saccarides.

A number of quantitative methods viz. Bartrands[106] and Somogyi's[107] are known for the determination of total sugars. Nowadays micro methods are commonly employed. The number of sugar groups attached to each aglycone in the purified glycoside can be determined after careful hydrolysis of each component (Ca 0.2 mg.). The resulting aglycone may be estimated spectrophotometrically and the sugars either one of the several micro-methods (given later) or by the anthrone reagent. The anthrone[108] method

dispenses with the step of hydrolysis and is specially useful for glycosides containing rhamnose since low concentrations of the sugar gives erratic results with the Somogyi's[109] reagent. A number of methods are known for the quantitative determination of reducing sugars, while for non-reducing sugars there are a few one. A list of some of the colorimetric and titrimetric methods is given below.

Colorimetric Methods:

(i) The Nelson colorimetric method for reducing sugars (copper sulphate solution and arsenomolybdate solution)[110]. Duff, and Eastwood[110] and Laidlaw and Reid[111] have successfully applied the method of Nelson[112] to sugars eluted from paper.

(ii) Determination of reducing sugars and furanoside non-reducing sugar by benzidine and acetic acid[113].

(iii) Determination of reducing and non-reducing sugars. (Phenol-sulphuric acid method)[114].

(iv) Anthrone sulphuric acid method[108,115].

(v) Aniline phthalate method[116].

(vi) A direct colorometric method for reducing sugars (alkaline triphenyl tetrazolium halide as spray reagent). The method is unique as coloured spot is eluted from the paper [117].

Titrimetric Methods:

(i) Determination of saccharides by hot periodate oxidation[118].

(ii) Somogyi's copper method[119].

Spot intensity measurements:

The method differs from other methods in that it does not require elution of the spot. (Direct measurement of spot intensity after reaction with silver nitrate, using standard photovolt electron-transmission densitometer[119].

(d) Study of the Striochemical Nature of the Glycosidic Union.

The steriochemical problem of the glycosidic linkage is readily revealed by the study of the behaviour of glycosides towards a particular enzyme. The action of enzyme is entirely specific. The enzyme emulsin attacks the β-glycosides only while the α-isomer is hydrolysed by maltase. Studies of the optical rotation of the glycoside can also be used to determine the steriochemical nature.

The determination of the positions of sugar residues:

The classical method for establishing the position of attachment of the sugar residue in a new flavonoid glycoside involves the methylation of free hydroxyl groups, followed by acid hydrolysis to remove the sugar residue. During hydrolysis of methylated glycosides fresh hydroxyl groups are introduced in each of the sugar units as well as in aglycone at the carbon atoms which were involved in the glycosidic linkages. Hence the isolation and melting point comparison of the resulting hydroxypolymethoxy compound with a sample of known constitution decides the positions of the glycosidic linkages. The partially methylated sugars are characterised by the methods described earlier.

The order of mono-saccharides in the case of biosides containing two different sugars is determined by separation and examination of the intermediate monoside resulting from partial hydrolysis[64,120,121].

Colour Reactions

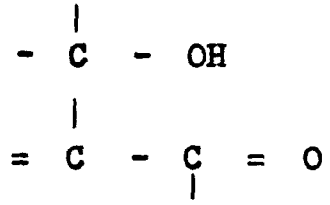
A number of colour reactions are used for detecting certain structural features among the hydroxy flavones. These tests, however, are not infallible[124].

Flavones and flavonols are readily detected in white or pale yellow tissues by the ammonia test. White tissues turn yellow and yellow tissues usually darken in colour on exposure to ammonia. The test is not specific for any single class of flavonoids. It is sensitive to flavones, flavanones, chalcones and xanthones. The addition of alkali to a crude or partially purified plant extract also serves as a substitute for ammonia.

Reduction of an alcoholic solution of a flavonoid with magnesium and hydrochloric acid as well as with sodium amalgam followed by acidification gives a pink to magneta colouration. The sensitivity of the magnesium-hydrochloric acid test is such that about 50 micrograms of quercetin can be detected with ease when this amount is present in approximately 0.5 cc of ethanol[125]. Asahina and Inubuse[126] first found that flavones may be reduced to anthocyanidine only in alkaline solution and flavonols in acid solution but flavanone in both acid and alkaline solutions. Brigg and Locker[127] extended the reaction and found that flavonols with a methoxyl group at C_3 and their 3-glycosides, in contrast with those with a free hydroxyl group at C_3 are reduced by sodium amalgam. Products isolated from this reduction and partially responsible for

the colour are flavylium salts, salts from 4-hydroxy flavones[128,129], and bimolecular products[130].

Wilson Boric acid Test[131]: Flavonols which contain a free 5-hydroxyl group react with boric acid in the presence of organic or mineral acids with the production of bright yellow colour. Neelkantan, Row and Venkateshwarlu[132] have specified the general structural requirements for a positive test as follows:-



Flavonols which lack the 5-hydroxy group (e.g.fisetin) do not respond[131] to this test, although Neelkantan[132] reports a positive test with 3,7-dihydroxy flavone. 5-Hydroxyflavanones do not respond to Wilson boric acid test[132,133].

Flourescence Tests:

Flourescence in flavones and isoflavones is brought out by certain solvents and ions. The phenomena is almost general in concentrated sulphuric acid but absent in alkali. This flourescence is not dependent on

the hydroxyl groups in the ring. Since flavone itself flouresces. It is characteristic of 3-hydroxyflavones but not of 5-hydroxy flavones[134,135]. Acetic anhydride brings out a flourescence with 5-methoxyflavones but not with 5-hydroxyflavones[136]. Flavanone gives blue flourscent spot[137] on paper under U.V.light when sprayed with magnesium acetate.

Ferric chloride colours:

The production of colours with ferric chloride is a general property of all classes of polyhydroxy flavonoid compounds. This property is of limited use in the examination of crude-reacting substances because of the non-specific nature of the reaction. However this is very helpful in determining the constitution of a pure substance and the location of the hydroxyl groups.

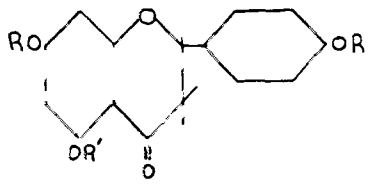
Both 5- and 3-hydroxy flavones give intense ferric chloride colours but the colours given by the 5-hydroxy compounds are almost invariably green[138] and occasionally brown[139] or purple[140] while the colour given by 3-hydroxy flavones is invariably brown[141]. 8-Hydroxy compounds also furnish either brown or green colouration with ferric chloride[127]. Green colour with ferric chloride indicates the absence of hydroxyl group in position ortho to the carbonyl group[142]. It was thus

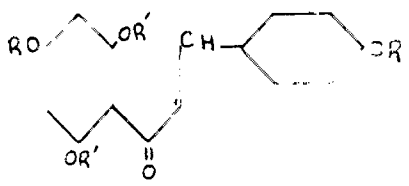
pointed out that the production of colours with ferric chloride solution is a property of 3,5-, or 8-hydroxy compounds but not of 6-, 7- or 4-hydroxy derivatives (Cf) also for 6-hydroxy flavones[143], for 7-hydroxyflavones[148], and for 4-hydroxyflavones[145].

Shimokoriyamo[146] has utilized magnesium hydrochloric acid and ferric chloride tests to establish the structures of four narangenin acetates as illustrated below. The acetylation of narringenin under different conditions can lead to the following four acetylated compounds.

XV	m.p.140-143°	Mg-HCl$^+$	$FeCl_3^-$
XVI	m.p.83-86°	Mg-HCl$^-$	$FeCl_3^-$
XVII	m.p.135-140°	Mg-HCl$^+$	$FeCl_3^-$
XVIII	m.p.95-100°	Mg-HCl$^-$	$FeCl_3^-$

These tests show that acetate XV and XVII are flavanones, XVII containing no free hydroxyl group, and acetate XVI and XVIII are acetates of the isomeric chalcone, XVIII being the completely acetylated tetrahydroxy compound.





XV R = AC, R' = H XVI R = AC, R' = H

XVII R = R' = AC XVIII R = R' = AC

Miscellaneous colour reactions:

A number of more colour reactions are reported in the literature for detecting certain structural features among flavonoids. The description of colours are subject to individual judgement, and, further, that the actual shade absorbed depends upon the concentration of the substance in the test solution. For example a very dilute solution of quercetin produces a rose-pink colour, more concentrated solutions produce crimson or magneta colours.

Marini-Bettolo and Ballio[124] have observed that the reaction of flavonoid compounds with antimony pentachloride in carbontetrachloride produces characteristic colours. These are similar in general to those produced with concentrated sulphuric acid.

5-Hydroxyflavones give addition compounds with stannic chloride. Flavonols are reported to be oxidised in cold alkali by air more easily than flavones. However this test is not completely reliable. An alcoholic solution of flavonols, which are hydroxylated at positions 5 and 8, gives a red precipitate with p-benzoquinone (Gossypetone reaction[148]) and is believed to be specific, for 5-, 8-, hydroxy flavonols.

(C) Absorption Spectra:

Ultra-violet Spectra:

The absorption spectra of flavonoid compounds have been studied extensively. It is mostly the Japanese School with Shibata and Kimtski[149], Tasaki[150], Hattori[151] and Hayashi[152] who have done a lot of work on spectral aspects of these pigments and have connected their particulars to their chemical constitution. Data on flavones have also been recorded by Lajos and Gerendas[153], Grinsbaumowna and Marchlewski[154] and above all by Skarzynski[155]. Aronoff[156] has made a critical study of the results of all the authors already cited and is well reviewed by Sannie and Sauvain[157].

The application of spectral data to the identification or structure determination of naturally-occuring flavonoid substances requires the use of pure samples in order that the observed absorption maxima may be relied upon when compared with reported values. Consequently it is usually not feasible to perform spectral measurements on direct plant extracts.

Skarzynski[155] has found the presence of two characteristic bands in the molecule of benzopyrone nucleus or chromone at approximately 3000A° and between 2000-2500°A. It has also been noted that the presence of a phenyl group at position 2 has got no effect on the fundamental spectra. Also the spectra of hydroxy and methoxy chromone do not differ from the spectra of corresponding flavones.

The flavone in itself presents well characteristic two independent bonds, one at 2975A° (Band I) and the other at 2500A° (Band II). It is probable that in the rest there exists a third band near 2000A° but it is not present in the usual spectra without a special technique. It is to be remarked that the attribution of these bands as specific of the benzopyrone nucleus is not absolutely certain[156]. On the one hand the bands are present at 2000 and 2500A° in the spectra of benzene and γ-pyrone, on

the other hand spectra of 2-hydroxy hydrochalcone is comparable to that of flavanone with the maximum at 2500 at 2300Å°. Therefore it is not the structure of benzopyrone nucleus responsible for the characteristic spectra of flavones but the presence of an analogous nuclear system which is present or is formed by chelation. Thus it is actually the form of resonance O- or p-responsible for it.

Following table III[64,155,158,160] shows the maxima of each band with the value of log ε .

T A B L E - III

Ultra-Violet Absorption Spectra of Flavone Derivatives

Flavone	max.	log ε
Flavone	297.5;250	4.20; 4.07
3',4'-di OH	345; 245	4.28; 4.17
5,7-di OH (chrysin)	330; 270	3.90; 4.42
5,7-di OAc	302.5;255	4.43; 4.18
5-OH-7-OMe (tectochrysin)	330; 270	3.88; 4.40
Apigenin-7-apioglucoside (apiin)	341; 267	4.29; 4.17
Apigenin-7-glucoside	335; 268	- -
Apigenin-7-rhamnoglucoside	335; 270	- -
5,7,4'-tri OH (apigenin)	340; 265	4.31; 4.25
5,7,4'-tri OMe	325; 265	4.33; 4.25

(Table III continued)

Flavone	max.		log ε		
Luteolin-7-glucoside	350;	259	-	-	
Luteolin-7-glucoside	350;	259	-	-	
5,7,3'-tri OH (luteolin)	355;	258	4.28;	4.22	
5,7,3',4'-tetra OAc	300;	258	4.35;	4.30	
3-OH (flavonol)	347.5;	305; 239	4.04;	3.83;	4.14
3,5,7-tri OH(galangin)	360;	267.5	4.07;	4.23	
3,7,3',4'-tetra OH(fisetin)	370;	315; 252.5	4.43;	4.22;	4.33
3,5,7,2'-tetra OH (datiscetin)	360;	262.5	3.99;	4.14	
3,5,7,4'-tetra OH(kampferol)	370;	310 ; 267.5	4.28;	-	4.12
3,5,7,3',4'-penta OH(quercetin)	375;	258	4.32;	4.32	
3,5,7,2',4'-penta OH(morin)	380;	263	4.15;	4.22	
3,5,4'-tri OH-3',7-di OMe (rhamnazin)	375;	255	3.27;	4.37	
5-OH-3,7,3',4'-tetra OMe	252;	269; 254	4.34;	4.29;	4.37
3,5,6,7,3',4'-hexa OMe	335;	240	4.42;	4.37	
3,5,7,8,3',4'-hexa OMe	351;	271; 252	4.33;	4.33;	4.34
3,5,6,7,3',4'-hexa OH (quercetagetin)	361;	272; 259	4.34;	4.15;	4.34
Quercitrin	352;	260	4.24;	4.35	
Rutin	361;	310; 258	4.28;	3.96;	4.35
Isoquercitrin	360;	310; 258	4.32;	4.01;	4.41
Hyperin	362,5;	312; 258	4.31;	3.97;	4.38
Quercimeritrin	374;	257	4.39;	4.42	
3,5,7,3',4'-penta OAc	300;	253	4.27;	4.32	
5,7-di OH-3-OMe-3',4'-methylenedioxy	353;	269; 255	4.28;	4.21;	4.32
3,5,7-tri OMe-3',4'-methylenedioxy	340;	263; 250	4.32;	4.21;	4.35
5,7,4'-tri OH-3,3'-di OMe	360;	268; 256	4.31;	4.24;	4.31
5,4'-di OH-3,7,3'-tri OMe	360;	268; 257	4.33;	4.24;	4.32
4'-OH-3,5,7,3'-tetra OMe	345;	263; 251	4.34;	4.22;	4.32
6-OH-3,5,7-tri OMe-3',4'-methylenedioxy	337;	272; 245	4.35;	4.07;	4.24
6-OH-7 OEt-3,5-di OMe-3',4'-methylenedioxy	337;	272; 244	4.38;	4.19;	4.25
Ternatin	368;	273; 258	4.28;	4.29;	4.33
Meliternin	351;	272; 253	4.29;	4.27;	4.36
Melinternatin	336;	269; 248	4.41,	4.11;	4.25
Melisimplexin	336;	235	4.29;	4.30	

The usual technique involving elution of appropriate portions of the paper chromatogram was impossible in some cases as the substance was eluted with boiling water or boiling alcohol. A similar difficulty has been reported by Gage and Wender[160]. Elution may be avoided and time saved by direct measurement of the absorption spectrum on the paper. Bradfield and Flood[66] measured the ultra-violet spectra of some of the compounds on paper chromatogram.

It is well known that the ultra-violet absorption spectra of the ions of many polar-substituted compounds are markedly different from the spectra of the compounds themselves, usually the main band having shifted 20 or more towards the red and increase in intensity. Except for flavonols and their 3-glycosides, the normal ultra-violet spectra of most flavone and flavonol glycosides are similar to those of the parent aglycone[150], it was felt that the spectra of the ions might afford an elegant method of differentiating such compounds which has been obtained in two small a quantity, for the normal chemical methods to be applied. It is evident that the position of attachement of sugar in flavone glycosides can be determined from the spectra of the ions of either the compounds themselves or the partial methyl ethers and the corresponding aglycones obtained from them on a micro-scale[121] by methyla-

tion and subsequent hydrolysis. This has been illustrated beautifully by the spectrophotometric study[122] of apigenin and its, 5,7-,5,4'-and 7,4'-dimethyl ethers first in ethanol and then in 0.002M sodium ethoxide in absolute ethanol. The spectra of two apigenin mono-glucosides (7- and 4'-glucosides) have also been compared.

Acetylation of phenolic hydroxyl groups substantially nullifies their effects upon the absorption, a polyacetoxyflavone having an ultra violet absorption spectrum very similar to that of flavone itself, a polyacetoxychalcone having a spectrum similar to that of flavone itself, a polyacetoxychalcone having a spectrum similar to that of benzalacetophenone, and a polyacetoxyaurone resembling benzalcoumaranone. Flavanones are colourless compounds and absorb at comparatively short wavelengths. Since hydroxylation in the 2-aryl group have very little influence on the positions of maximum absorption of flavanones, the use of absorption spectra in this class of compounds is largely limited to classification as to type and can not be usefully extended to the analysis of structural details.

The absorption and flourescence spectra of substituted chromones[161] flavonols[162], benzylidene coumaranones[163] and the absorption spectra of dihydroflavonols[164] have been studied and discussed.

Infra-red spectra

The infra-red spectra came into use recently and nowadays it is utilised more and more usually for the qualitative and quantitative analysis of the unknown substances. Hergert and Kurth[165] have measured the infra-red frequencies of a series of flavanones and related compounds in Nujol or perfluoro kerosene Mull.

The carbonyl and hydroxyl frequencies of this series of compounds are presented in the following table[165].

T A B L E - IV

Compound	Frequency Cm^{-1}	
	Carbonyl	Hydroxyl
Flavanone	1680	-
3',4'-Dihydroxy-	1665	3395,3105[a]
3',4'-Diacetoxy-	1762,1680	-
3',4',5',7-Tetrahydroxy-	1620	3260[a]
3',4',5,7-Tetracetoxy-	1763,1680	-
5-Hydroxy-3',4',7-trimethoxy-	1610[b]	-
3,3',4',5,7-Pentahydroxy-	1642	3510,3355[a]
3,3',4',5,7-Pentaacetoxy-	1764,1703	-
3,3',4',5,7-Pentamethoxy-	1649	-
3,5-Dihydroxy-3',4',7-trimethoxy-	1606[b]	3380
Acetophenone	1687	-

(table IV contd.)

Compound	Frequency Cm^{-1} Carbonyl	Hydroxyl
2-Hydroxy-[c]	1635	-
2'-Acetoxy-	1762,1678	-
2-Benzoxy-	1736,1684	-
2-Methoxy-[c]	1649	-
4-Hydroxy-	1638	3100[a]
4-Acetoxy-	1763,1685	-
4-Methoxy-[c]	1657[b]	-
4-Methoxy-2-hydroxy-	1615	-
2,4-Dihydroxy-	1620	3260,3150[a]
2,4-Dimethoxy-	1643	-
2,4-Diacetoxy-	1764,1688	-
Chalkone(benzalacetophenone)	1659	-
2',3,4-Trihydroxy-	1621	3280[a]
2'3,4-Triacetoxy-	1762,1661	-
2',3,3',4,4'-Pentahydroxy-	1619[b]	3250[a]
2',3,3',4,4'-Pentabenzoxy-	1744,1656	-
Flavone		
3,3',4',5,7-Pentaacetoxy-	1763,1640	-
3,3',4',5,7-Pentahydroxy-	1654	3290[a]
3,3',4',5,7-Pentamethoxy-	1627	-
3,3',4',7-Tetramethoxy-5-hydroxy	1657	-
3',4',5,7-Tetrahydroxyflavone-3-rutinoside	1655	3270[a]
3,3',4',5,8-Pentahydroxy-	1655	3340
3,3',4',5,8-Pentaacetoxy-	1764,1645	-

[a] Broad band, not sharply defined. [b] Exact position in doubt because of interference by phenyl band at 1605-1590 cm^{-1}.

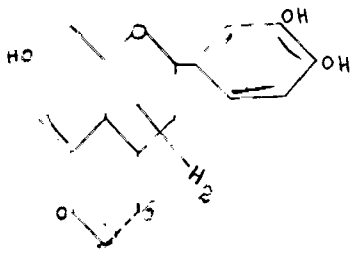
[c] Liquid.

It is well known observation in infra-red spectroscopy that conjugation of ethylenic double bonds, or a carbonyl group and double bonds cause shift from the normal position to a longer wavelength. Thus an unconjugated carbonyl group in acetone shows a band at 1718 cm^{-1} while conjugation with one phenyl group as in acetophenone lowers the frequency to 1687 cm^{-1} and conjugation with two phenyl groups as in benzophenone lowers the frequency to 1655 cm^{-1}.

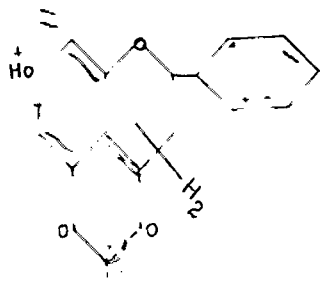
Flavanones:

Unsubstituted flavanones shows a band at 1680 cm^{-1}. Introduction of hydroxyl groups in the 3' and 4'- positions cause the carbonyl frequency to shift to 1665 cm^{-1}. Acetylation of these groups cause a shift back to 1680 cm^{-1}, a frequency identical with that of unsubstituted flavanones.

Introduction of hydroxyl groups into the 5- and 7-positions shifts the carbonyl frequency 1620 cm^{-1}. It is concluded that the following are important resonance structures having hydrogen bonding between the 5-hydroxyl and the keto group.



XIX



XX

3',4',7-Trimethoxy-5-hydroxy derivative has a carbonyl frequency of 1610 cm^{-1} and shows no band attributable to a hydroxy group.

Acetylation of the 3,3',4',5,7-pentahydroxy flavone shifts the carbonyl band from 1642 to 1703 cm^{-1}. Since the acetoxy derivatives have a higher carbonyl frequency than unsubstituted flavone and 3',4',5,7-tetracetoxy flavone, it appears likely that the 3-substituent is responsible for the effect.

Chalcones:

Unsubstituted chalcone shows a carbonyl band at 1659 cm^{-1} which is due to conjugation with a phenyl group and an aliphatic double band. Introduction of a hydroxyl group in the conjugated chelated 2'-position lowers the carbonyl frequency to about 1620 cm^{-1}. Acetylation causes a return to the original unsubstituted position.

Flavones:

The flavone derivatives do not show marked lowering of carbonyl frequency when a hydroxyl group is present in the 5-position. Acetylation of the hydroxyl group decreases, rather than increases the carbonyl frequency. The 5-hydroxy group is involved in chelation is apparent since the OH band is absent in 5-hydroxy-3,3',4',7-tetramethoxy flavone. Introduction of a methoxyl group in the 5-position causes a shift to 1627 cm^{-1} for 3,3',4'5,7-pentamethoxy flavone. This value is 22 cm^{-1} lower than that of the corresponding flavone derivative. The lowering is due, at least partially, to increased conjugation, which is not possible in the flavanone.

As the infra-red spectra in Nujol or perfluorohexane mulls have significant changes in the absorption spectra due to the interaction in the solid phase, Shaw and Simpson[166] have measured, the infra-red absorption spectra, in carbontetrachloride solution of a number of flavanones and flavones, in the carbonyl stretching frequencies, are recorded in the following table.

T A B L E - V

Compounds	C=O frequency cm^{-1}	Compounds	C=O frequency cm^{-1}
Flavanones:		3',4'-dimethoxy	1647
		3-hydroxy	1619
(Unsubstituted)	1695	5-hydroxy	1652
7-methoxy	1685	3,5-dihydroxy	1638
7,4'-dimethoxy	1686	3-hydroxy-7-methoxy	1621
5-hydroxy	1648	3-hydroxy-3'-methoxy	1619
		3-hydroxy-3',4'-dimethoxy	1616
Flavones:		3-hydroxy-7,3'-dimethoxy	1616
		*3-hydroxy-7,4'-dimethoxy	1611
(Unsubstituted)	1649	5-hydroxy-7-methoxy	1659
7-methoxy	1640	5-hydroxy-3'-methoxy	1645
3'-methoxy	1655	5-hydroxy-4'-methoxy	1649
4'-methoxy	1653	5-hydroxy-7,3'-dimethoxy	1677
7,3'-dimethoxy	1638	*7,3',4'-trimethoxy	1638
7,4'-dimethoxy	1646		

* These compounds were not sufficiently soluble to give carbonyl absorption of greater than 20%. The reported figures may therefore be inaccurate.

Flavanones: It is seen from above table that introduction of a methoxy group in the 7-position of the nucleus causes a frequency shift of 10 cm^{-1}.

Flavones:

The above table lists the differences between the carbonyl stretching frequency of substituted flavones and the corresponding flavanones. The unchelated compounds

show the expected frequency decrease resulting from the increased conjugation of the carbonyl group. The difference between the carbonyl frequencies, of 5-hydroxy flavone and 5-hydroxy flavanone is due to the unusual properties of the former chelated system.

In the interpretation of infra-red spectra the analyst should take into consideration all the empirical constants established by the numerous workers throughout the world. The infra-red spectroscopy is a technique which has a great future and permits to elucidate the structure of the natural or synthetic molecule and differentiate between the isomers which are difficult to study by other methods.

Polarographic Analysis of Flavonoid Compounds.

Flavones, Flavanones and chalkones show well defined polarographic reduction waves. Engelkemeir, Geissman, Crowl and Friess[167] and Hinreiner[125] applied the polarographic method to the analysis of plant materials. The method does not appear to be a useful one for structural characterisation since the half wave potential for structurally related compounds differ by very little. At pH 7.7, Engelkemeir[168] et al found the half-wave potentials (vs. the saturated calomel electrode) for

quercetin, quercitrin and apigenin to be - 1.62, - 1.58, and - 1.63 volts respectively.

The potential value of the polarographic method appears to lie in the quantitative estimation of the total flavone content of plant material, by the measurement of the height of the reduction wave at the flavone potential. Fries[125] used the method for the measurement of the "flavone" content of ten carnation (Dianthus caryophyllus) genotypes.

The limitations of the polarographic method are the requirements for careful removal of interfering reducible substances (e.g. oxygen) and the difficulty in estimating wave-heights when waves are poorly defined, as is sometimes the case. It is probable that if a solution of an unknown flavone or flavone mixture is sufficiently carefully purified to allow polarography to be used, other methods of analysis (e.g. chromatography on paper followed by spectrophotometric measurements) would be more reliable and equally convenient.

Degradation of the Aglycone.

Kostanecki, Herzig and Perkin[168] applied the technique of degrading the aglycone into its simpler components and then comparing them with the original substances.

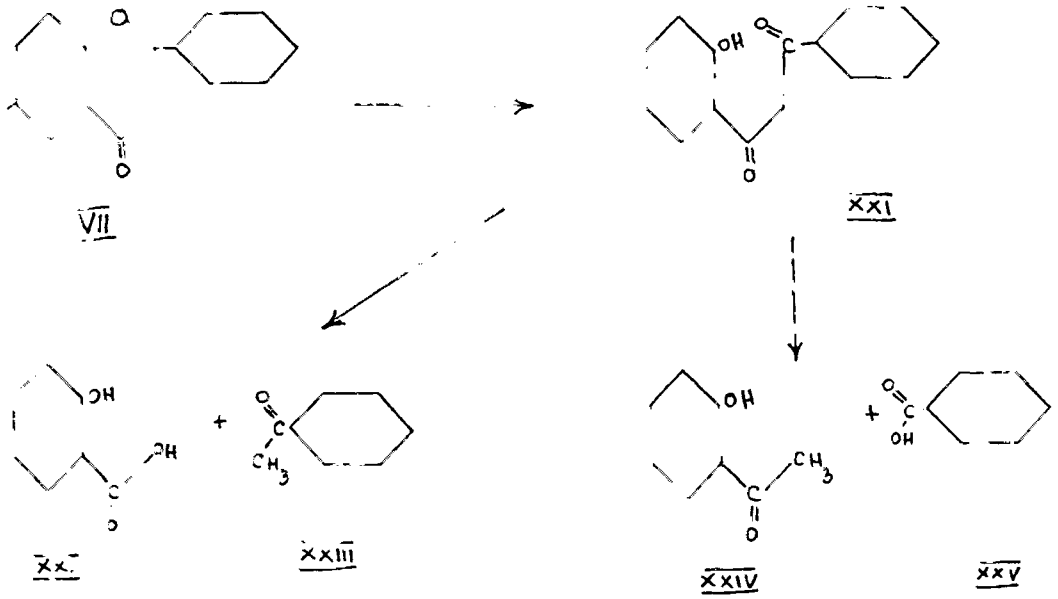
(i) Alkaline fusion:

By fusion with alkali the pyrone nucleus is disintegrated into a phenol and an aromatic acid. Thus with flavone we have phenol and benzoic acid.

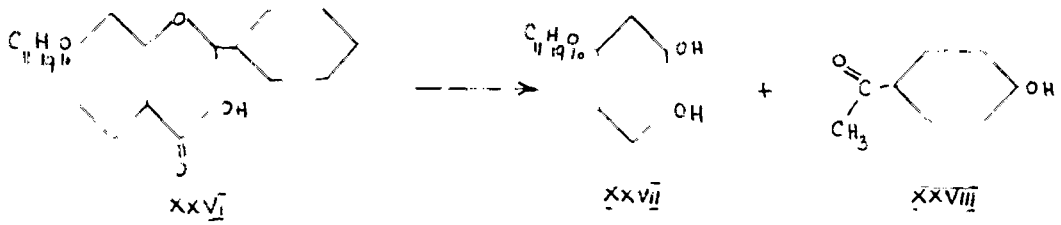
The method has been widely used for establishing the structure of various hydroxylated aglycones[169,70,71] but is found fruitless in case of methylated aglycones. The methoxyl group is knocked out during the process[170].

(ii) Alkaline hydrolysis:

By boiling the flavone with a concentrated solution of alkali (25%, 5 hours) the degradation of the molecule takes place in stages and may adopt two courses.



When a glycoside is boiled with alkali (25%, 5-hours), the molecule undergoes similar degradation without any change of the carbohydrate position. The glycoside apiin is thus degraded to p-hydroxy acetophenone and a phloroglucinol glycoside.



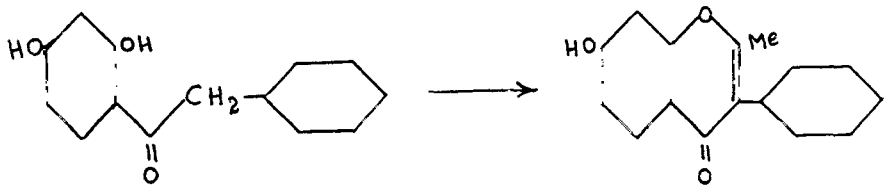
XXVI XXVII XXVIII

Methods introduced by Paul Karer[172-173] for establishing the precise nature of the phenyl residue in position 2 and the point of linkage of the sugar residue are of great importance. Prior to Karrer's work the position of the methoxyl residue into anthocyanidin group, peonidin, malvidin and hirustidin was not known, since the concentrated alkali employed to degrade the pigment also removed the methoxyl groups. Karrer's degradation of the sugar-free pigment, with dilute barium or sodium

hydroxide (10%) in an atmosphere of hydrogen, which yielded the phenolic acid with the methoxyl group intact was therefore, a significant advance.

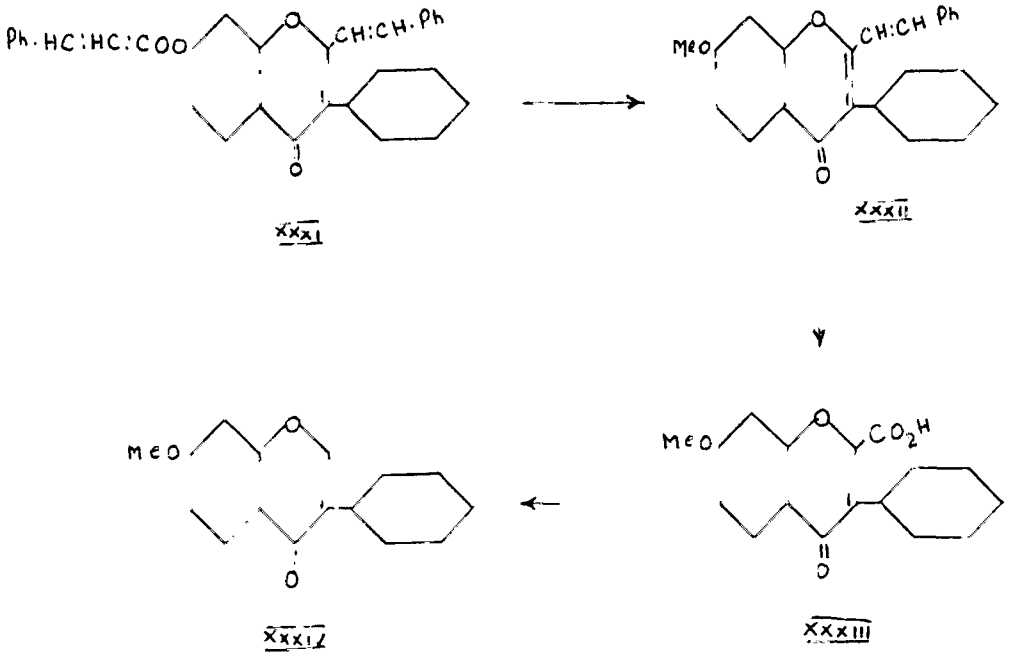
Synthesis of Isoflavones

The synthesis of isoflavone (3-phenyl chromone) with substituents (alkyl or aryl) in position 2 presented no difficulty, but since this position is never substituted in naturally occuring compounds, such synthetic methods are of value only if the substituent can be subsequently removed. Baker and Robinson[174] showed that condensation of 2,4-dihydroxydeoxybenzoin XXIX with acetic anhydride and sodium acetate followed by hydrolysis gave 7-hydroxy-2-methyl isoflavone XXX. Deoxybenzoins derived from phloroglucinol gave 5,7-dihydroxyisoflavone.



XXIX XXX

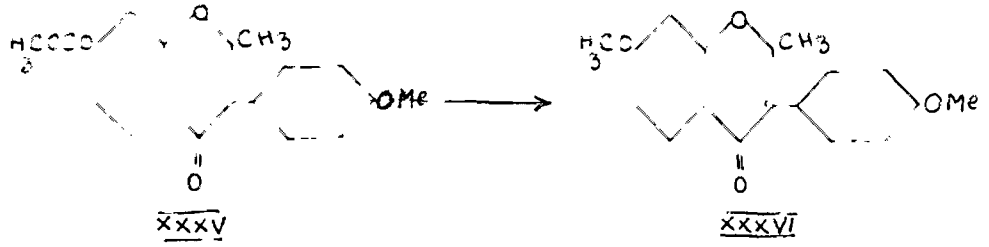
It was further noted that the condensation also proceeded with benzoic anhydride and sodium benzoate and the product obtained was 2-phenyl isoflavone. The interaction of cinnamic anhydride and sodium cinnamate with 2,4-dihydroxydeoxybenzoin gave 7-cinnamoyloxy-2-styryl isoflavone XXXI. This compound was converted into the 7-methoxy derivative XXXII. The degradation of 7-methoxy-2-styryl compound by oxidation with potassium permanganate in pyridine solution followed by thermal decomposition of the resulted 7-methoxy isoflavone-2-carboxylic acid XXXIII. gave, ultimately, 7-methoxy isoflavone XXXIV.



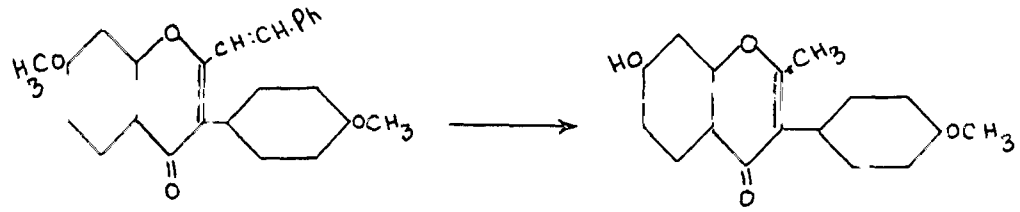
XXXI XXXII XXXIII XXXIV

Inspite of numerous attempts at that time to devise a more practicable synthesis of isoflavones bearing no substituents in position 2, it was found almost indispensible to proceed through the 2-styryl isoflavones and the isoflavone carboxylic acids derived in small yields by oxidation.

The difficulty, however, of preparing 2-styryl isoflavone, was reduced by condensing 2-methyl isoflavone with benzaldehyde. This method which is due to Baker, Robinson and Simpson[175] avoids the experimental difficulties arising from the use of derivative of cinnamic acid. Thus the condensation of 2,4,dihydroxy-4'-methoxy-deoxybenzoin with acetic anhydride sodium acetate gave 7-acetoxy-4'-methoxy-2-methyl isoflavone XXXV which after deacetylation and methylation XXXVI was condensed with benzaldehyde in presence of sodium ethoxide. The resulting 2-styryl derivative XXXVII on usual degradation and demethylation gave daidzein XXXVIII in 5% yield. The method was also applied successfully for the synthesis of 4-baptigenin[176]. This method like the original one



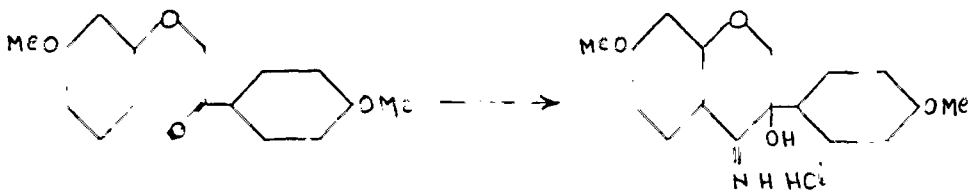
XXXV XXXVI



XXXVII XXXVIII

suffers from the limitations of poor yields in oxidation, and since hydroxy groups must be protected before oxidation it is limited to compounds in which the final demethylation will not cleave required alkoxy group.

Baker, Pollard and Robinson[177] evolved a synthesis of 7-methoxy isoflavone which avoided oxidation altogether. The condensation of m-methoxyphenol with phenacylbromide gives m-methoxyphenoxy acetophenone XXXIX, which was converted into the cyanohydrin. This compound on treatment with zinc chloride and hydrogen chloride in ether underwent an intramolecular Hoesch reaction, and the ketimine hydrochloride XL on hydrolysis yielded 3-hydroxy-7-methoxy isoflavone which was degraded to 7-methoxy isoflavone by sulphuric acid. This method in

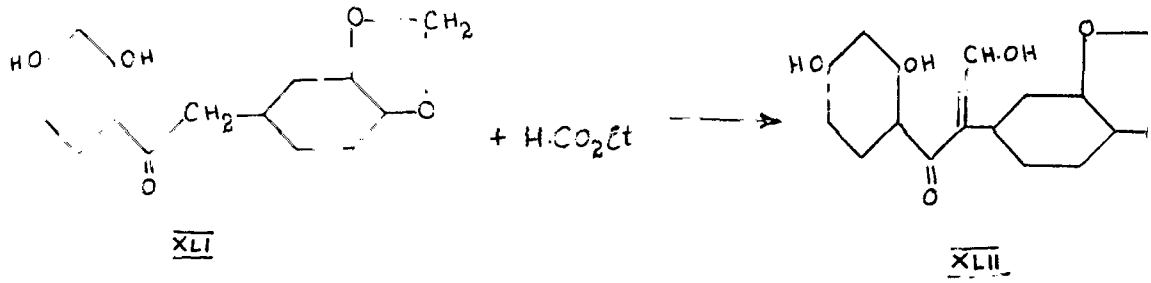


XXXIX XL

which the yields are good at all stages, was later employed by Spath and Lederer[178] for the preparation of ψ-baptigenin. The synthesis of irigenin trimethyl ether by Baker, et al[179] could not be accomplished by the application of this method.

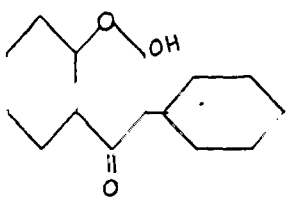
Spath and Lederer[178] in 1930 condensed 2,4-dihydroxy-3',4'-methylenedioxydeoxybenzoin with ethyl formate in presence of sodium at 100°C in a sealed tube and obtained ψ-baptigenin in a very small yield. The method was later applied by Wessely, et al[180] to the synthesis of daidzein and fomononetin but the yield in each case was poor. The ethyl formate-sodium synthesis was greatly improved in 1934 by Mahal, Rai and Venkataraman[181] who carried out the condensation at 0° and obtained daidzein and ψ-baptigenin in yields of about 30% each from the corresponding deoxybenzoins. The use of this method is limited to deoxybenzoins having only one free hydroxyl group required for ring closure but there are reports for the successful synthesis of a isoflavone from deoxybenzoin having more free hydroxyl groups. Shriner and Hull[182] described the preparation of 8-methylgenistein from a trihydroxy deoxybenzoin, but later workers including the present author have not been able to reproduce the results.

The mechanism of ring closure between a deoxybenzoin and ethyl formate has been much discussed. Spath and Lederer[178] suggested the following course:



XLI XLII

The active methylene group of the deoxybenzoin undergoes formylation followed by enolisation XLII. The unsaturated triol loses water to form an ether linkage on treatment with mineral acid. No such intermediates, however, were isolated by them or by Nahal, Rai and Venkataraman[181], who considered that the reaction proceeded directly to isoflavone. There are cases, however, in which intermediates of the molecular composition postulated above have been isolated. Wolfrom and coworkers[183] in 1941 isolated four such compounds to which they gave the 2-hydroxy-isoflavanone structure XLIII.



They showed that these compounds in every case lost a molecule of water on treatment with glacial acetic acid to yield an isoflavone. It is to be noted that the usual methods of removing water of crystallisation did not expel any water.

Harper[184] in 1942 using derritol and elliptol methyl ether and Mehta et al[185] in the synthesis of isogenistein and 8-methyl isogenistein have also recorded the same observation of the formation of 2-hydroxyisoflavanone with ethyl formate-sodium synthesis. However Venkataraman et al[181], Robertson, Suckling and Whalley[186] and Rahman et al[187] obtained isoflavone directly under conditions which might have been expected to give the intermediate hydroxy isoflavanone if they had been formed. It is interesting to mention here the observation of Narasimbhachari et al[188] who have found that the use of methyl formate in isoflavone condensation invariably yields 2-hydroxyisoflavanone. This finding has shortly after been confirmed by the work of Whalley[189]. Consideration of the actual structures of the compounds, the condensing reagents and the experimental conditions in these apparently in-consistent experiments does not greatly help in exploring why only in <u>some cases</u> stable intermediates with ethyl formate are formed and why the same intermediates are <u>invariably</u> formed with methyl formate.

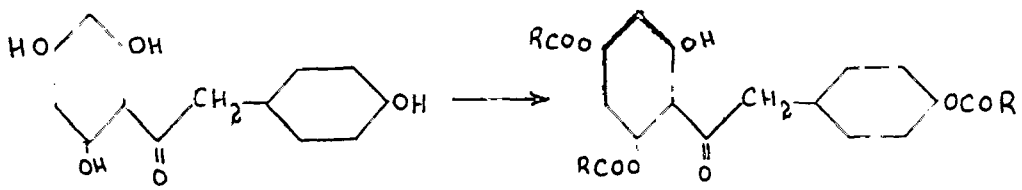
Sathe and Venkataraman[190a] in 1949 used ethyl-orthoformate in pyridine containing a little pipperidine as a condensing agent in the synthesis of 7-hydroxy-isoflavone. Prunitin[190a] and 6-hydroxy-5,7-dimethoxy[190b] have also been synthesised from the corresponding deoxy-benzoins by the use of this reagent. This reagent also suffers from the disadvantage that it is not generally applicable to polyhydroxydeoxybenzoins.

Although the ethyl formate-sodium synthesis has been of great value, its failure with deoxybenzoin contain-ing several free hydroxyl groups has been a severe limita-tion. In 1949 an elegant ethoxalyl chloride synthesis was discovered in which ring closure can be carried out with compounds containing several free hydroxyl groups. Baker et al[191] reported that the 2-carbon atom could be furnished by ethoxalyl chloride and that isoflavone -2-carboxylic esters were formed in good yields. A number of papers have appeared on the successful use of this reagent[192]. The new synthesis has got the distinct advantage of the direct preparation of polyhydroxy and partially alkylated hydroxy isoflavone in high over all yields. Dealkylation, which is here avoided, is sometimes accompanied by reorien-tation of a substituted group from position 8 to 6 in 5-hydroxy flavones and has been observed also in the

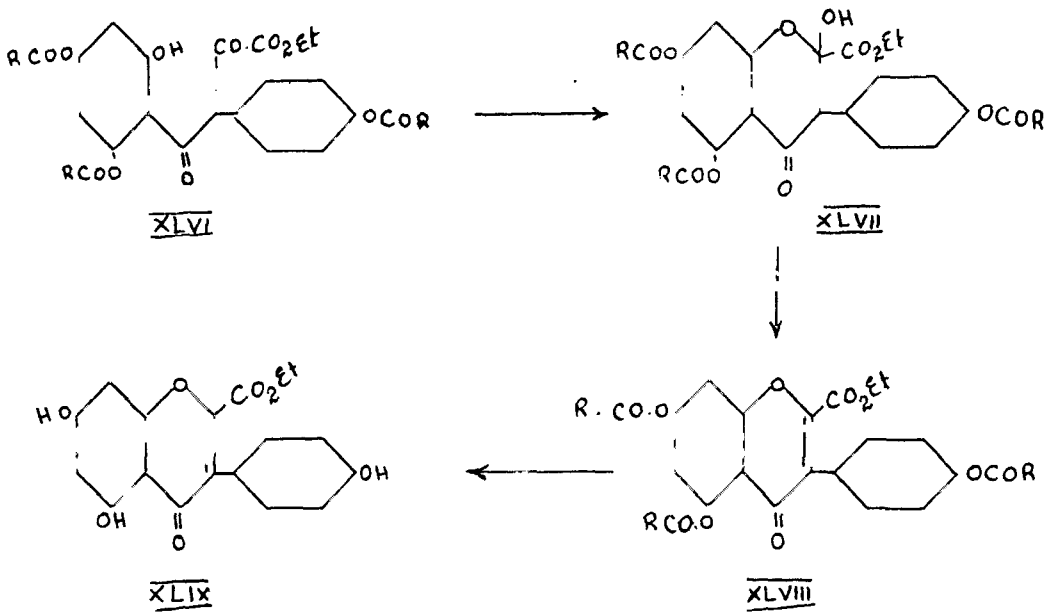
isoflavone series. The value of the method was shown by the preparation at the Middlesex hospital of 120 gms of genistein in 50% yield from the corresponding deoxybenzoin.

The deoxybenzoin containing in all n free phenolic group is treated with n + 1 equivalent of ethoxalyl chloride in pyridine and left over night, water then precipitate the ethyl isoflavone-2-carboxylate. Hydrolysis with sodium carbonate is followed by decarboxylation which occurs at a little above the melting point.

Baker et al[191] suggested the following probable mechanism: (a) ethoxalylation of all phenolic hydroxyl groups except one ortho to the carbonyl group giving XLV; (b) C-ethoxalylation of the reactive methylene group of the deoxybenzoin XLVI; (c) cyclisation to the 2-carbethoxy-2-hydroxy isoflavanone XLVII; (d) loss of a molecule of water to give the 2-carbethoxyisoflavone XLVIII; (e) removal of the ethoxalyl group by reaction with dilute acids giving XLIX.



XLIV XLV



When the ethoxalylation was carried out in pyridine and boiling benzene, the isoflavone esters were in most cases isolated directly, but in very low yields.

2-Hydroxy isoflavanone, have been recognised as intermediates in the ethylformate isoflavone synthesis[93,183,184] (some cases) and methylformate[189,191,193] synthesis (invariably). Baker et al[192] have, however, found that in the case of 2-hydroxy-4:6-dimethoxydioxybenzoin the primary product of reaction with ethoxalyl chloride is 2-hydroxy 5,7-dimethoxy isoflavanone, which loses water when treated with acetic acid to give 5,7-dimethoxy isoflavone.

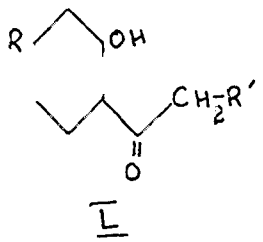
Methoxalyl chloride[194] has also successfully been used in some cases for the synthesis of isoflavones but the scope and limitations have not been fully explored.

Various sources of C(2)-atom in isoflavone, which would permit of their ready synthesis from 2-hydroxy-deoxybenzoin have been investigated[195]. It has now been found that formamide or preferably formanilide[196,197] can in some instances supply the necessary C(2) atom with yields of isoflavone upto 60%. The technique is simple, the amide and the deoxybenzoin are heated together for about 30 minutes, normally the isolation of isoflavone presents little difficulty. It is necessary to protect hydroxyl groups in the deoxybenzoins except that in the 2-position. The reaction, while useful in particular cases, does not displace as the method of choice the ethoxylyl chloride process which can be employed with polyhydroxydeoxybenzoins without protection of the hydroxyl groups.

The possibility of a thermally induced Baker-Venkataraman transformation of O-acyloxyacetoarones into the corresponding o-hydroxy-1:3-diketone or flavone[198,199] has also been extended for the synthesis of isoflavones[196]. It has now been found that 2-acyloxydeoxybenzoins, when

heated to 250° give the corresponding 2-substituted isoflavone, in some instances in satisfactory yield. This reaction parallels the ready manner in which these compounds undergo the Baker-Venkataraman base-catalysed transformation[200,201,202] which may also be brought about thermally. As in the formamide method, polyhydroxydeoxybenzoin do not react well.

Recently Farkas[203] described a method in which the C(2)-atom is obtained by the action of hydrogen chloride on zinc cyanide. It has been shown that β-keto-aldimines of type LI are obtained from various derivatives of deoxybenzoin (L) (I), R = OH, OMem R' = Ph, P-OH C_6H_4) by the above reagent.



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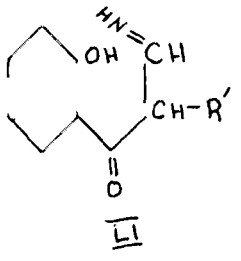
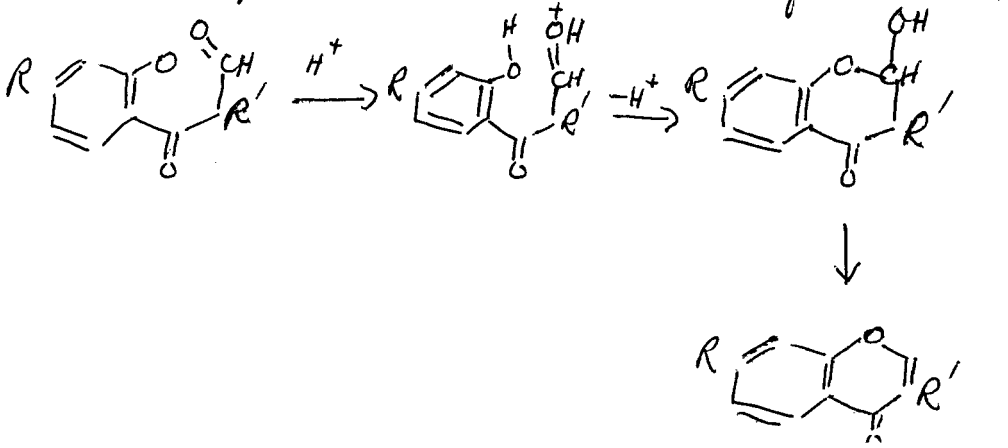
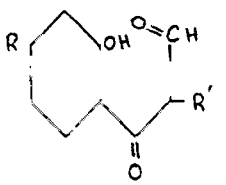


LI

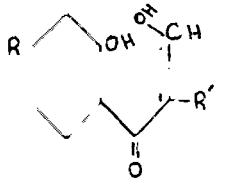
The ring closure occurs as follows:



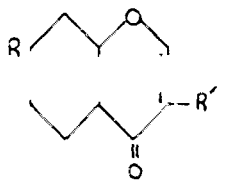
Hydrolysis of (LI) affords compounds (LIa) and (LIIb), respectively. Compounds of type(LII-b) undergo ring closure with loss of water and formation of the corresponding isoflavones (LIII).



LIIa



LIIb



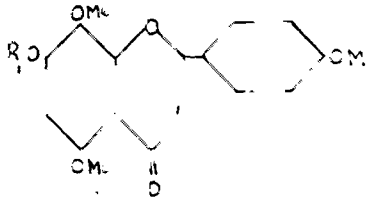
LIII

It is not necessary to protect hydroxyl groups during the synthesis, a fact which gfeatly facilitates the preparation of isoflavones.

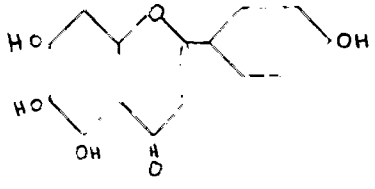
Isomerisation in Isoflavones.

In the synthesis of most flavones and related compounds, demethylation is an essential step and hydriodic acid is the reagent most commonly used. During hydriodic demethylation, rearrangement of a 5,7,8- to 5,6,7-trihydroxy flavone was observed by Wessely and Moser[204] who obtained 5,6,7,4'-tetrahydroxy flavone (LV)

(Scutellarein) from 7-hydroxy-5,8,4'-trimethoxy flavone (LIVa).



LIV

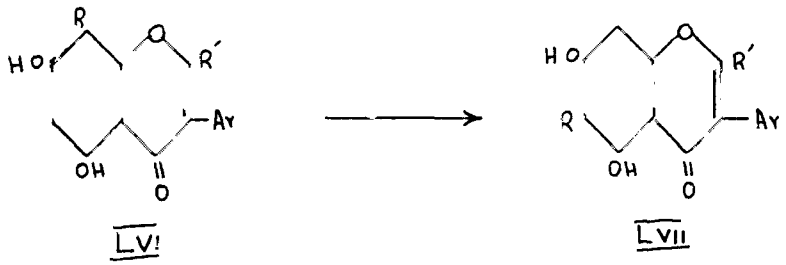


LV

a) R_1 = H b) R_1 = Me

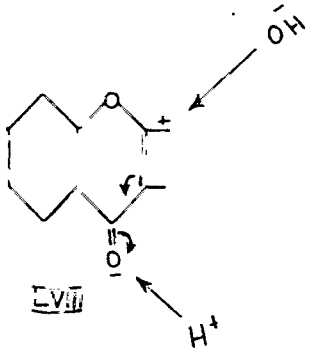
The view was later confirmed by Wessely and Kallab[205] who found that contrary to the result of Hattori[206] 5,7,8,4'-tetramethoxy flavone (LIVb) on treatment with hydroiodic acid suffers isomeric change to scutellarein (LV). This important type of rearrangement of a 5,8- to 5,6-orientation during demethylation under the influence of hydroiodic acid is known as Wessely-Moser rearrangement and was for the first time observed in flavones. The rearrangement is not only confirmed to flavones but is exemplified by most of the compounds related to flavones e.g. flavonols, flavanones, chromones, chromonols, xanthons and isoflavones.

The rearrangement of 5,8- to 5,6-dihydroxy flavones and chromones under the conditions of demethylation with hydrobromic or hydroiodic acids is well established and is due to the hydrolytic opening of the pyrone ring and closure in the alternative direction involving the hydroxyl group initially in position 5. As expected such changes occur with the isoflavones e.g. 5,7,8-trihydroxy-2-methyl isoflavone (LVI, R = OH, R' = Me, Ar = Ph) when boiled for 8 hours with hydrobromic and acetic acids gives the 5,6,7-isomer, (LVII; R = OH, R' = Me, Ar = Ph)[207]. It has now been found[208] that demethylation of 5,7-dihydroxy-8,3',4',5'-tetramethoxy isoflavone (LVI; R = OMe, R' = H, Ar = 3,4,5-trimethoxy phenyl) gives 5,6,7, 3',4',5'-hexahydroxy isoflavone (irrigenol) (LVII; R = OH, R' = H, Ar = 3,4,5-trihydroxy phenyl). The conditions of the reaction may be the deciding factor in controlling whether or not the rearrange-

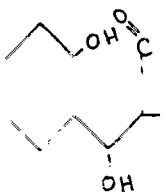


LVI LVII

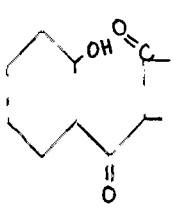
ment occurs. Thus 5,7,8-trimethoxy isoflavone and its 2'-methyl derivative[209,210] methylgenistin[189], isogenistin[211] have been demethylated with hydriodic acid without change of orientation. Mukerji et al[212] have advanced an explanation as to why flavonols, chromonols and isoflavones do not undergo isomerisation under the usual conditions of demethylation. According to them the electrophilic activity of the 2-position is considered to be mainly responsible for this reaction and the ring opens out to form a diketone or its equivalent. The hydroxyl group in the 8-position may have some effect in encouraging the ring fission but does not seem to be absolutely essential since this isomeric change is found to take place even in 6- and 8-methyl compounds. But the presence of substituents hydroxyl (methoxyl) and phenyl in the 3-position is of importance. They seem to inhibit ring opening and this is attributed to their capacity to reduce the electrophilic acitivty of the 2-position by an electrometric mechanism.



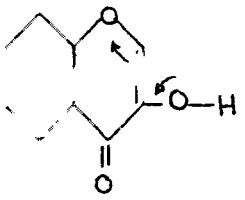
LVIII



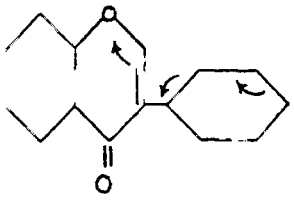
LIX



LX



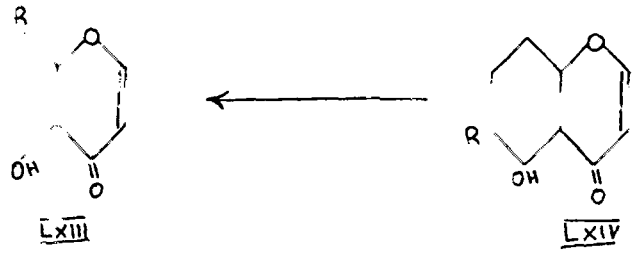
LXI



LXII

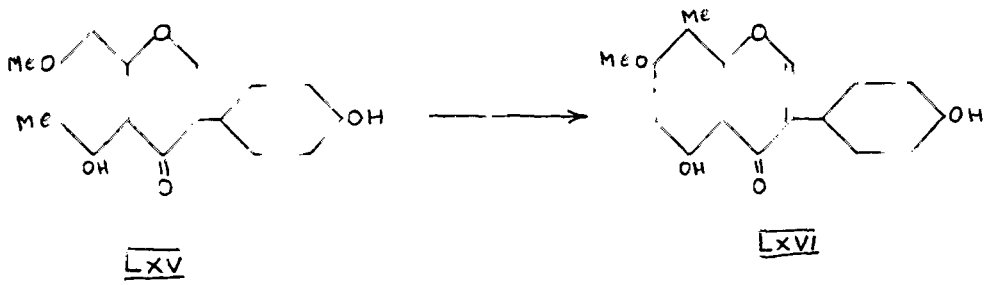
Wheeler et al[213] and Baker et al[208] have shown that under drastic conditions, flavonols, chromones and isoflavones also can be made to undergo the isomeric change. However, the explanation of Mukerjee et al[9] is still valid because under ordinary conditions the change does not take place and hence resistance to ring fission is definite though not absolute. But Whalley's[214] report of isomerisation of methyl isogenistein by boiling with "stablised" hydroiodic acid only for 45 minutes, and the observations in these laboratories for methyl isogenistein and 5,7-dimethoxy-8-methyl isoflavone (unpublished results) undergoing isomerisation exactly under conditions as prescribed by Seshadri and Varadayan[211] are not in agreement with the explanation of Mukerjee et al[212].

The reverse change i.e. the rearrangement of compounds of type LXIV to those of type LXIII has not been reported in any series of such compounds with the exception of only one example in chromone series[212,215].



LXIII LXIV

The reverse type of change for the first time has been reported[216] in isoflavone series, when 5-hydroxy-7,4'-dimethoxy-6-methyl isoflavone LXV, on demethylation with hydroiodic acid, furnished two distinct products isolated after remethylation i.e. (a) 5-hydroxy-7,4'-dimethoxy 6-methyl isoflavone LXV and (b) 5-hydroxy-7,4'-dimethoxy 8-methyl isoflavone LXVI.

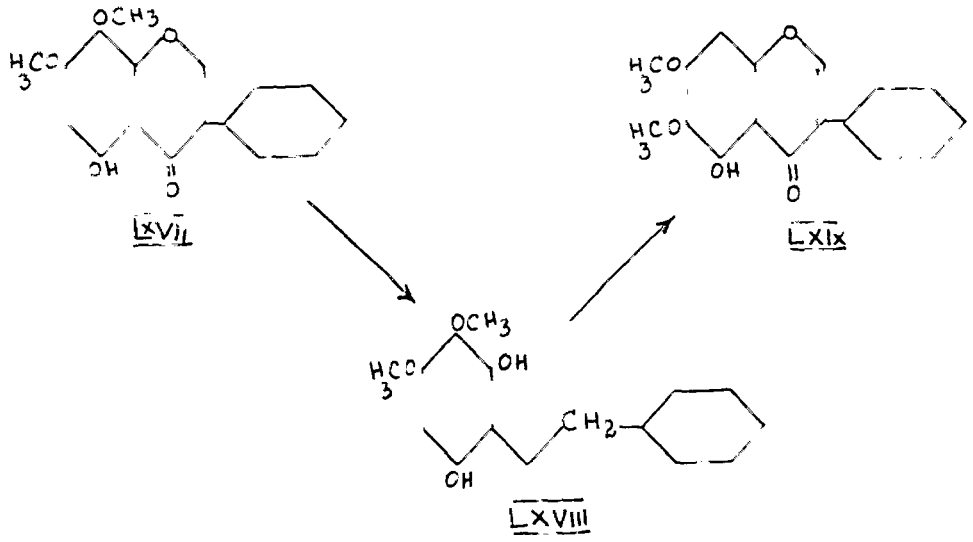


LXV LXVI

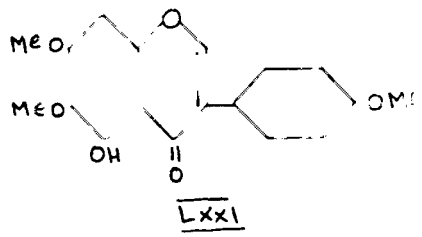
Whalley[214,216] has observed that in case of 6,7,2'- or 5,7,4'-trimethoxy-8-methyl isoflavone, demethylation with aluminium chloride in dry benzene yields a mixture of products having 8- and 6-C-methyl orientation. Wheeler et al[213] have suggested that as no rearrangement has yet been observed during demethylation by aluminium chloride,

the production of some 6-isomer by the reagent might be ascribed to the direct migration of methyl residue rather than to ring opening followed by a ring closing in an alternate position.

In all the above discussions isomeric changes took place in acid media. Alkaline solution could not be used because decomposition sets in. A special case was observed when the change took place to some extent in alkaline medium. It has been shown that 5-hydroxy-7,8-dimethoxy isoflavone LXVII undergoes fission with alcoholic alkali and when the resulting phenyl benzyl ketone LXVIII is resubjected to isoflavone condensation the alternative structure with 5,6,7-rearrangement (5-hydroxy-6,7-dimethoxy isoflavone LXIX) is produced[217]. In the course of their study it was noted that even during the alkaline fission a small quantity of the isomeric isoflavone was formed. This constitutes an example of isomeric change in alkaline medium.

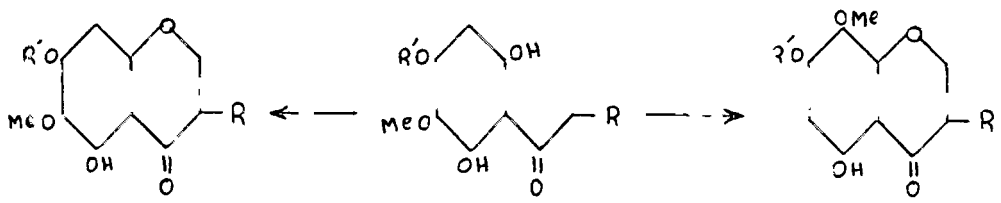


Another problem of orientation has been encountered in the ethoxalyl chloride isoflavone synthesis. The ketone(LXX; R = p - MeO.C_6H_5, R' = Me) when submitted to ethyl formate-sodium synthesis gives 5-hydroxy-6,7,4'-trimethoxy isoflavone (LXXI; R = p - MeO. C_6H_4, R' = Me),



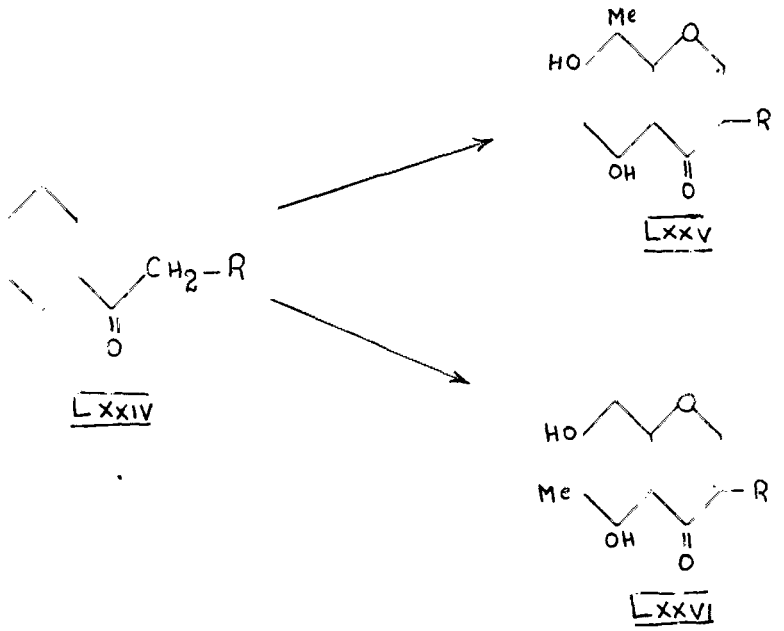
LXXI

tectorigenin dimethyl ether[218], in which the 6-hydroxyl group has been involved in closure when, however, the ketone (LXX; R = p - OH. C_6H_4; R' = H) is submitted to ethoxalylation process, the final product is 5,7,4'-trihydroxy-8-methoxy isoflavone (LXXIII; R = p - OH. C_6H_4; R' = H) an isomer of tectorigenin[208] resulting from cyclisation with the alternative hydroxyl group.



Hence in such case, whilst the ethyl formate method gives rise to compounds with the 5,6,7-orientation, the ethoxalytic process gives the 5,7,8-orientation.

Recently it has been observed that the C-methyl deoxybenzoins on ethoxalylation yield a mixture of isomeric isoflavones having 5,7,8-,and 5,6,7-orientations.



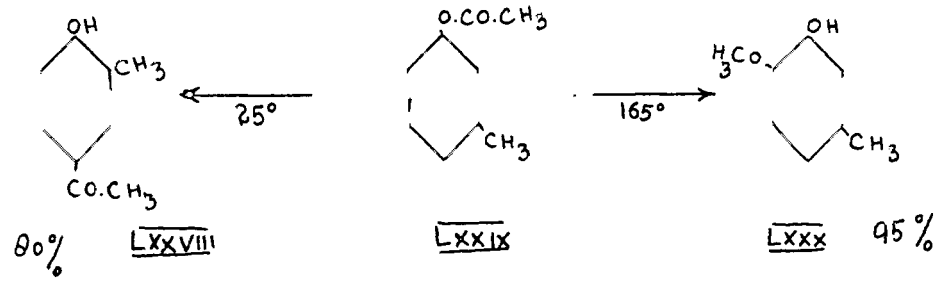
This is illustrated by taking the example of 2,4,6-trihydroxy-3-methyl deoxybenzoin (LXXIVa; R = pH)[219] and its 2- and 4'-methoxyderivatives LXXIVb, LXXIVc. When a mixture of isoflavones having 5,7,8- and 5,6,7-orientation has been obtained in each case.

DEOXYBENZOINS

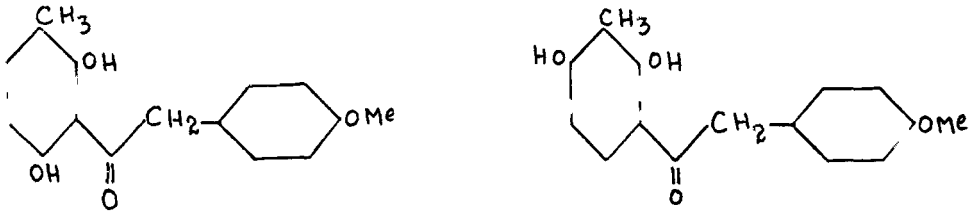
No useful synthesis of isoflavones has appeared which does not require an o-hydroxydeoxybenzoin. A number of methods are available for the preparation of deoxybenzoins of the required type. The most useful and widely used method being the Hoesch reaction[220]. A phloroglucinol or recorcinol reacts with a phenylacetonitrile and hydrogen chloride in dry ether, to give a ketimine hydrochloride, which is hydrolysed to the deoxybenzoin by boiling with dilute mineral acid. This method of choice suffers from the limitations that it cannot be used for hydroquinone[220] and pyrocatechol[220] nuclei. Other methods of importance which may be mentioned here are (i) Friedel-crafts acylation (ii) Fries rearrangement and (iii) Neneki's reaction.

These methods have got the disadvantage of giving rise to mixtures of o-, and p-isomers, thus necessitating their separation. The mixture of hydroxy ketones, obtained during the course of Fries rearrangement, may frequently be readily separated by virtue of the fact that the o-isomer due to chelation, is volatile with steam, whereas the p-isomer is not. Further more, the composi-

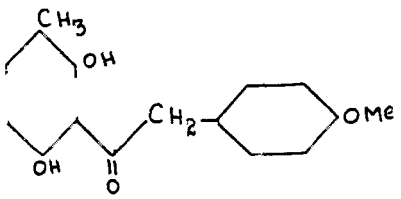
tion of the product may sometimes be largely determined by control of the reaction conditions. High reaction temperature, for example, favours the production of the o-hydroxy ketones relative to p-isomer. A striking example of the phenomenon is afforded by the Fries rearrangement of m-cresyl acetate.



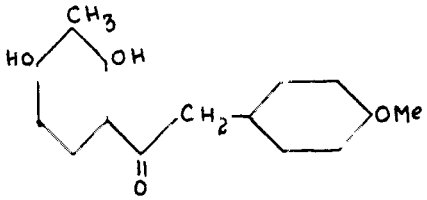
Although the extreme variation of the product composition is not always observed, it is generally true that the formation of o-isomer is favoured by high reaction temperature. Other experimental variables, such as the solvent and proportion of the catalyst, also somewhat influence the product composition.



The synthesis of deoxybenzoin of certain required orientation inaccessible by the conventional methods, require blocking off the active position in the phenol nucleus. For example, an attempt to obtain 2,6-dihydroxy-4'-methoxy-3-methyl deoxybenzoin LXXXI by the condensation of methyl resorcinol with p-methoxyphenyl acetonitrile (Friedel-Crafts acylation) may result in the formation of 2,4-dihydroxy-4'-methoxy-3-methyldeoxybenzoin (LXXXII).



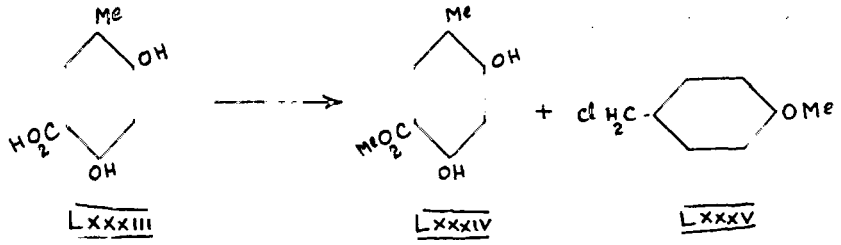
LXXXI

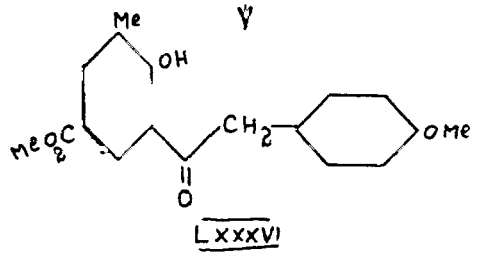


LXXII

The deoxybenzoin[221] LXXXI has now successfully been synthesised by (a) carboxylation of methyl resorcinol (Kolbe.Schmidt method) (b) preparation of its methyl ester LXXXIV; (c) Friedel-Crafts acylation with p-methoxyphenyl acetonitrile LXXXVI and (d) finally hydrolysis and decarboxylation of 2,6-dihydroxy-4'-methoxy-5-methoxy-

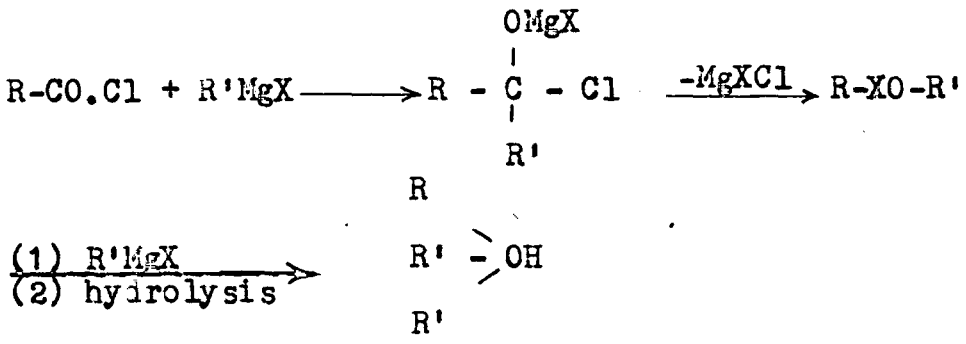
carbonyl-3-methyldeoxybenzoin LXXXV. Due to the well known inhibiting influence of meta directing group (carbmethoxy in the presence case) on Friedel-Crafts acylation, it is brought about successfully by keeping the reaction mixture for 12 days at room temperature. Various steps of the reaction are schematically shown below.





The fact that the Trichloro-w, trifluoro acetophene[222] undergoes haloform reactions with the production of the corresponding acid and halo form, has also been successfully exploited for the intrcduction of carboxyl group in phenols. The carboxy group, as mentioned earlier may be esterified and the phenolic ester may now be subjected to conventional method for the preparation of ketones of desired orientation.

Organometallic compounds have also found immense use in the synthesis of deoxybenzoin. Grignard reagents react with acid chloride to give a ketone as the initial product. The ketone can, ofcourse, react with more Grignard reagent to give a tertiary alcohol.



The elimination of Mg X Cl occurs in the dry ether solution in which Grignard reactions are customarily carried out, so that the ketone is present in the reaction mixture at all times. Success of the reaction as a preparative method for ketones depends upon the fact that the Grignard reagent reacts somewhat faster with an acid chloride than with a ketone. Fairly satisfactory yields of ketones may be obtained when an ether solution of the Grignard reagent is added dropwise to the acid chloride ("Inverse" Grignard addition) so that Grignard reagent is never present in excess.

Jenkin's[223] modification of original Bies[224] method for the preparation of aliphatic ketones has been of great value in the synthesis of deoxybenzoins. The substituted benzamides react with Grignard reagent to give stable complexes which may be subsequently decomposed with the formation of ketones. The success of the method depends upon the fact that the primary addition product of the Grignard reagent to the amide is fairly stable and remains as such in the reaction mixture. Since ketone is not produced until addition of acid in the work-up procedure, there is little danger of the formation of tertiary alcohol. Amides of the type R - Co.NH_2 and R - Co.NHR' contain active hydrogen and decompose the Grignard reagent. This possibility is over-come to a large extent by the use of excess of Grignard reagent (4 times) or better by using the amides of the type R - Co-N-R_2. The sluggish nature of the amido group towards Grignard reagents necessitates considerable increase in reaction time (48 hours). The scope of the method is limited by the condition that no other function capable of reacting with a Grignard reagent may be present in the molecule, since the amido function as already pointed out is one of the least reactive towards addition of Grignard reagent.

Other organo metallic compounds viz. those of zinc[224] and cadmium[226] have also been used and found superior to Grignard reagents. The success of organo cadmium compounds specially as contrasted with Grignard reagent depends upon their negligible reactivity towards the ketone function. There is, thus, no necessity for 'increase' addition of the reagent. Yields of ketone by the organo cadmium method are high, frequently of the order of 70-80% or still better. Whilst there can be little doubt that the method employing organo cadmium compound is convenient for preparation of the simpler type of ketones, its general application is inevitably limited by the usual restriction accompany the use of Grignard reagents. Thus the use of sterically hindered acid chlorides or the organo cadmium compounds containing large alkyl or aryl groups result in the inhibitation of the normal reaction and initiating the abnormal one.

The methods including the use of general β-keto ester synthesis derived by Breslow, et al[227] have also been found unsatisfactory owing to low yields at one or more of the stages involved viz., the synthesis of β-keto ester, mono-, or di-akylation and eventual ketonic hydrolysis, sometimes under severe conditions[228].

The method involving the use of β-keto ester was modified by R.E. Bowman[229]. It consists of the preparation of (a) ethoxy magnesium malonic ester (b) acylmalonic ester (c) acid-catalysed acidolysis followed by spontaneous or subsequent thermal decarboxylation of the keto acid to the ketone. The various steps are shown below:

$$RCO.CH.(CO_2Et)_2 \underset{R'CO_2H}{\overset{H^+}{\rightleftharpoons}} R'.CO_2Et + RCO.CH(CO_2H).CO_2Et$$

$$\xrightarrow{-CO_2} R.CO.CH_2.CO_2Et \underset{R'CO_2H}{\overset{H^+}{\rightleftharpoons}} R'.CO_2Et + R.CO.CH_2.CO_2H$$

$$\xrightarrow{-CO_2} R.CO.CH_3$$

Failure to extend this reaction to the fully substituted compounds $R.Co.CR(CO_2Et)_2$ demonstrated the need of an entirely new approach to the problem. The hydrogenolytic fission of benzyl ester appeared to offer an alternative method of developing a general ketone synthesis as follows[229,230]:

$$CH_2(CO_2.CH_2\ Ph)_2 \longrightarrow R'.CH(CO_2.CH_2.Ph)_2 \longrightarrow$$

$$R'C.Na\ (CO_2.CH_2\ Ph)_2 \xrightarrow{RCO\ Cl} R.CO.CR'(CO_2.CH_2\ Ph)_2$$

LXXXVII LXXXVIII

$$\xrightarrow{H_2\text{-}Pd} R.CO.CR'\ (CO_2\ H)_2 \longrightarrow R.CO.CH_2\ R' + 2CO_2$$

LXXXIX XC

The method thus consists in the preparation of sodiobenzyl ester LXXXVII in situ from the corresponding ethyl ester by ester interchange with benzyl alcohol in benzene solution, the sodium enol acting as catalyst for the interchange and the reaction being forced to completion by removal of the ethanol formed as its azeotrope with benzene. The next stage is the coupling of the sodio derivative LXXXVII with the requiste acid chloride, catalytic debenzylation of the resulting keto ester LXXXVIII, and finally thermal decarboxylation of the acid LXXXIX to the ketone LXC.

The use of boron-fluoride[231,232] phenyl acetic acid complex has also been mentioned in the synthesis of deoxybenzoins. This method has been found of value when the conventional methods of acylation either fail altogether or give a poor yield. The usefulness of the method is illustrated by the preparation of following difficultly accessible ketones:

1. 2-hydroxy-4,4',5,6-tetramethoxydeoxybenzoin.
2. 2-hydroxy-4',5-diethoxy-4,6-dimethoxydeoxybenzoin
3. 2,4'-dihydroxy-4,5,6-trimethoxydeoxybenzoin
4. 2,5-dihydroxy-4,6-dimethoxydeoxybenzoin.

Other methods of some importance which may be mentioned here are:

(i) Benzoin reduction[233,234].

(ii) Fission of the pyridinium (nucleus) to form enamines then to deoxybenzoins[235].

(iii) Use of dinitrogen tetraoxide.[236]

D I S C U S S I O N

Argemone mexicana

Argemone, a genus of prickly herbs includes about twelve species. Argemone mexicana (Eng. Prickly poppy, Mexican poppy; Hind Bharband, Satiyanashi) is the only species found in India[237]. It runs wild all over the country and has now become a troublesome weed.

The yellow juice which exudes when the plant is injured, has long been used in India as a medium for dropsy, jaundice and cutaneous affections. It was also considered as diuretic[227]. The seeds yield 22.36% of a nauseous, bitter non-edible oil. The adultration of edible mustard oil with Argemone oil is probably responsible for outbreaks of epidemic dropsy[238,239]. Its presence is detected by the development of orange red colour, when concentrated nitric acid is added to the oil or its mixtures[237]. The oil is used as an illuminant and lubricant, and in medicine for external application in skin diseases. Mixed with dyeing oils such as linseed oil it may be used in paint industry[237]. The oil cake can-not be used as cattle fodder because of residual oil. However it could be used as a fertilizer.

The mixed fatty acids consist chiefly of oleic (22%) and linolic (48%) acids with some palmito-oleic (about

3%) and ricinoleic (about 10%) acids[240]. The plant is also reported to contain berberine and protopine as alkaloidal contents[241].

So far as known to the authors, no work appears to have been done on the colouring matter of the bright yellow flowers. The preliminary examination indicated the flower rich in flavonoids and, therefore, the present investigation was undertaken.

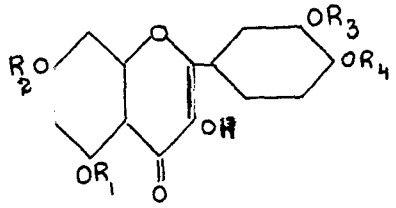
Dried flowers of Argemone mexicana were extracted successively with petroleum ether (40-60°) and ethanol (95%). The alcoholic extract was distilled under diminished pressure, the residue was taken up with water and extracted repeatedly with n-butyl alcohol. The combined n-butanol extracts, on leaving over-night, deposited a yellowish brown solid which was filtered off and marked "A". The filtrate on recovery of the solvent left behind a powdery residue of reddish brown colour. The residue was dissolved in hot water and the aqueous solution on treatment with neutral and basic lead acetate yielded a yellow bulky precipitate marked "B" and an orange precipitate labelled as "C" respectively.

The lead salt "B" was suspended in a large volume of ethanol and decomposed by passing hydrogen sulphide gas. The filtrate on distilling of ethanol left behind a yellow solid which was taken up in dry acetone. The acetone solution was passed through a column of magnesium trisilicate. A highly flourescent band (U.V.light) was removed during the course of washing the column with acetone. This was found to be non-flavonoid in nature. The other two flavonoid bands (U.V.light) were eluted with ethylacetate-water. The paper chromatographic examination of both the fractions revealed their non-homogenity. They were finally purified by paper chromatography on Whatman No.3 filter paper according to the method of Ice and Wender[63]. The two fractions after repeated crystallisations from methanol give minute yellowish brown plates m.p.165-67° and yellow shining needles m.p.304-6°. The acetone solution of the solid obtained from "C" on similar treatment revealed only one band in ultra-violet light. This was eluted with ethyl acetate-water. On recovery of the solvent the residue crystallised from methanol in yellowish brown plates m.p.165-67°. It showed no depression in melting point on admixture with one of the products obtained earlier from "B".

The yellow solid "A" which separated on leaving n-butanol extract over night, was dissolved in boiling methanol. The yellow microscopic needles m.p.202-205° separated from methanolic solution. Repeated crystallisations from the same solvent raised the melting point to 208-10°. The homogenity of the product was established by chromatographic examination.

The free aglycone m.p.304-306° was found to be a flavonol as it gave a pink colouration on reduction with magnesium and hydrochloric acid[126] and a bright yellow colouration with Wilson-boric acid reagent[131]. The methanolic solution of the aglycone was not oxidised by pentamine cobaltrichloride[242] indicating the absence of two or more adjacent phenolic hydroxyl groups. Micro-Zeisel determination showed the presence of only one methoxyl group. Methylation of the aglycone with methyl sulphate yielded a compound that melted at 151-52° and showed no depression in melting point on mixing with an authentic sample of pentamethyl ether of quercetin. The above observations proved that the aglycone was a monomethyl ether of quercetin. A number of monomethyl ethers of quercetin XCI are described in the literature, these are 7-,5-,3'-, and 4'-monomethyl quercetin. The possibility of the present aglycone having a methoxyl group at

C(5) was ruled out as it did not show fluorescence in acetic anhydride[136]. A comparison of the melting points of aglycone and its acetate with those of known monomethyl ethers of quercetin and their acetates suggested its resemblence with isorhamnetin.



XCI

	Aglycones	m.p. (aglycone)	m.p. (acetate)
R_1, R_2, R_3, R_4 = H	Quercetin	313-14°	194
R_1, R_3, R_4 =H; R_2=CH_3	Rhamnetin	300	190-92°
R_2, R_3, R_4=H; R_1=CH_3	Azaleatin	301-2°	197-198.5°
R_1, R_2, R_4=H; R_3=CH_3	Isorhamnetin	302-5°	202-204°
R_1, R_2, R_3=H; R_4=CH_3	4'-methylether of quercetin.	256-58	202°

Briggs and Lockers[127] observation that the flavonols with a methoxy group at C(3) in contrast with those with a free hydroxyl group at this position are also reduced by sodium amalgam, the appearance of a pink colouration with this reagent in the present case

led us to believe that it may be a 3-monomethyl ether of quercetin (not hitherto reported). This possibility was ruled out when it was found that an authentic sample of isorhamnetin also produced pink colour with this reagent.

The aglycone was characterised as isorhamnetin with melting and mixed melting points with an authentic sample of isorhamnetin and its acetate. Further confirmation to its identity was furnished by ferric reaction, Rf value, co-chromatography and spectral evidences. The ultraviolet and infrared spectra of the aglycone were found to be superimposable with those of a standard sample.

The glycosidic nature of the products (m.p.165-67° and 208-10°) was evidenced by the positive Malisch test obtained after hydrolysis and by the formation of an osazone in each case. Both the glycosides gave positive tests with magnesium and hydrochloric acid and sodium amalgam followed by acidification, indicating their flavanone or flavonol nature (with C_3 blocked)[127]. The appearance of a yellow colour with Wilson-boric acid reagent[131] eliminated the possibility of the glycosides belonging to flavanone class. Both of them on hydrolysis gave the same aglycone m.p.304-306°, which was charac-

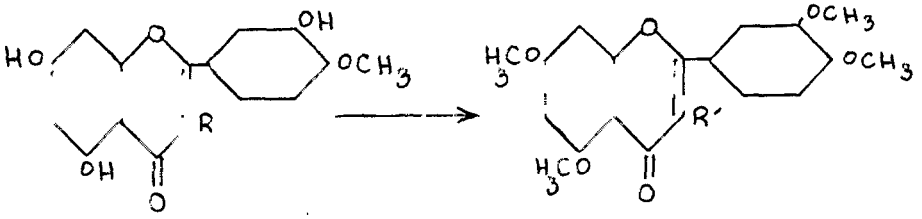
terised as isorhamnetin as described earlier. The sugar part in each case was found to be glucose by Rf value, co-chromatography and by the formation of osazone m.p.204-206°.

The possibility of uronic acids[77] as the carbohydrate moiety was also taken into account and it was found that uronic acids are not present as the sugar part of either of the glycosides. On the basis of the above colour reactions and examination of the products of hydrolysis the glycosides were identified as flavonol glycosides having isorhamnetin as aglycone and glucose as the carbohydrate moiety.

The position of the sugar residue in the glycosides was determined by methylation (methyl sulphate) followed by hydrolysis of the methylated glycosides. The partial methyl ethers obtained in both the cases were characterised by melting and mixed melting points with authentic samples and by the preparation of their acetates.

The complete methylation of the glycoside XCII m.p.165-67° gave an uncrystallizable oily mass XCIII which on hydrolysis yielded a product XCIV m.p.193°. It was characterised as 3',4',5,7-tetramethoxy quercetin[243]

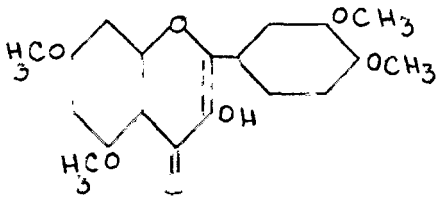
by the melting and mixed melting points with an authentic sample. The formation of the above tetramethyl ether of quercetin proved the attachment of the sugar residue at C(3) of the aglycone.



XCII XCIII

R = glucose residue

R' = methyl glucose residue

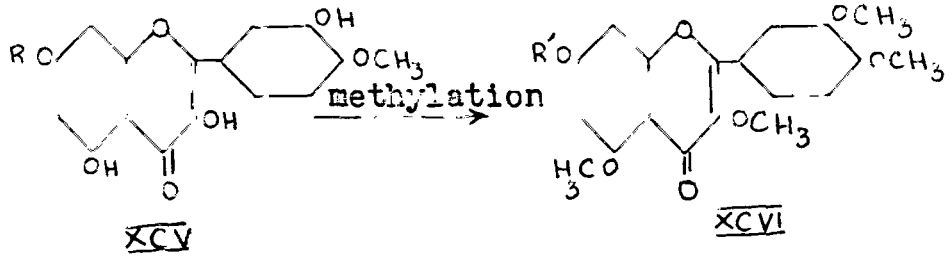


XCIV

The quantitative estimation of sugar by Somogyi's copper micro method[109] showed the presence of 1 mole of glucose per mole of aglycone. The aglycoside m.p.165-67° was, therefore, characterised as isorhamnetin-3-glucoside XCII.

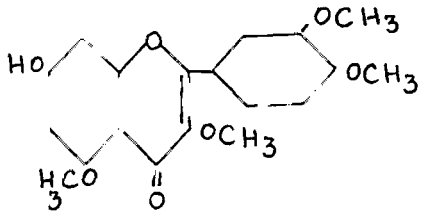
The completely methylated product XCVI from the glycoside XCV m.p.208-10° gave on hydrolysis a product

m.p.234-35°. It was characterised as 7-hydroxy-3',4',3,5-tetramethyl quercetin XCVII by melting and mixed melting points with an authentic sample. It gave an acetate m.p.174° (Cf.[244] 174-76°). The formation of 7-hydroxy 3',4',3,5-tetramethyl quercetin proved the attachment of glucose at C(7).



XCV XCVI

R = glucose residue
R' = methyl glucose residue



XCVII

The estimation of sugar by Somogyi's copper micro method[109] showed the presence of two moles of glucose per mole of aglycone. The glycoside m.p.208-10° was therefore identified as isorhamnetin-7-diglucoside.

Ervatamia coronaria

Ervatamia coronaria Stapf, E.Divaricata Linn., known as Chandni in Hindustani is a small ever green shrub with silvery grey bark and handsome foliage. The plant bears white flowers almost throughout the year. The flowers are sweetly fragrant at night and inodorous during the day. The plant is considered to be indigenous to India and is cultivated in gardens for its ornamental flowers.

The root is acrid and bitter, it is employed as a local anodyne and chewed for the relief of tooth ache. It is rubbed into a thin paste with water and administered as a vermicide[245]. It is also applied with lime juice to clear opacity of the cornea[245]. The root charcoal and the milky juice of the leaves are used in ophthalmia.

The bark of the stem and roots has been found to contain two alkaloids, tabernaemontanine ($C_{20}H_{26}O_3N_2$; m.p.208-10°) and coronarine[246] ($C_{44}H_{56}O_6N_4.2.5\ H_2O$; m.p.196-98°); a crystalline resin alcohol (m.p.180-81°), caoutchouc, resins, sugars and fatty matter (palmitic, ceratic and oleic acids)[246].

As no work appeared to have been done on the colouring matter of abundantly available white flowers the present investigation was undertaken.

Dried flowers of Crvatamia coronaria were treated successively with petroleum ether (40-60°) and ethanol. Theethanolic extract on concentration left behind a viscous dark brown residue, which was taken up with water. The aqueous solution was shaken repeatedly with n-butyl alcohol. The combined n-butyl alcohol extracts, on recovery of the solvent, left behind a reddish brown mass. It was dissolved in hot water and the insoluble material filtered off. The addition of a few cc of neutral lead acetate solution to the hot aqueous solution resulted in the separation of a dirty brown greasy precipitate which was removed by filtration and discarded. The clear filtrate on treatment with more quantity of lead acetate solution followed by the addition of ammonia gave a bulky orange yellow precipitate. The precipitate was filtered and washed several times with water. The precipitate was suspended in a large volume of ethanol and decomposed by passing hydrogen sulphide gas. The clear deep yellow filtrate on evaporation to dryness, under diminished pressure, left behind a non-crystallisable mass ~~of brown colour~~.

The amorphous brown material on chromatographic examination with three solvent systems revealed the presence of one major yellow flourescent spot and three minor spots of blue colouration (U.V.light). The acetone solution of the material was subjected to purification by colomn chromatography on magnessium trisilicate. The elution with ethyl acetate-water resulted in the separation of the single yellow flourescent band. The ethyl acetate solution on corcentration and leaving over night in an ice box separated into a powdery mass of light brown colour. Several crystallisations from methanol gave light brown needles m.p.222-24°. The homogenity of the product m.p.222-24° was established by paper chromatographic examination in a number of solvent systems. Further purification of the glycoside was accomplished by the preparation of its acetate in colourless needles m.p.158-60° and regenerating the glycoside by deacetylation. The product on crystallisation from methanol separated into light brown needles m.p.222-24° (Cf.Lit.[247] 223-24° for 3 glycoside of kaempferol).

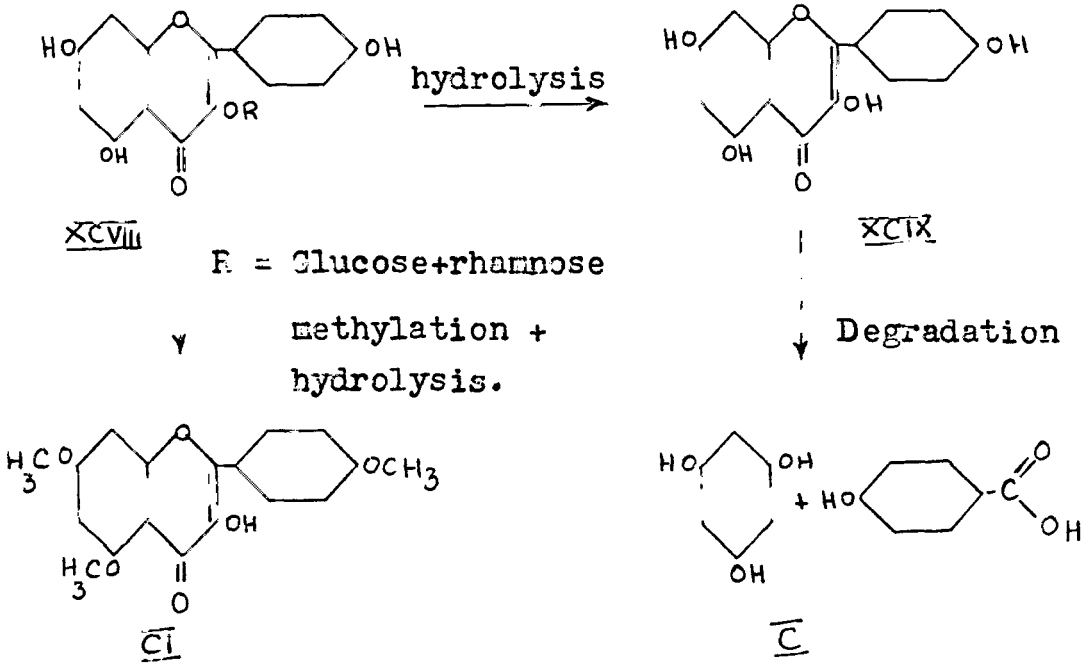
The glycoside on acid hydrolysis gave an aglycone which crystallised from dilute pyridine as light yellow needles m.p.276-78°. The aglycone was characterised as

kaempferol by co-chromatography with an authentic sample and by the comparison of Rf, value: 0.83 (n-butanol: acetic acid:water, 60:10:20) and 0.50 (acetic acid:water, 60:40). The aglycone showed no depression in melting point on admixture with an authentic sample of kaempferol. It gave an acetate m.p.180-82°. Its identity as kaempferol was further confirmed by micro degradation followed by the chromatographic examination of the fragments. The chromatogram on spraying with bisdiazotized benzidine revealed two spots indisdinguishable from those of authentic samples of phloroglucinol and p-hydroxybenzoic acid, Rf (phenol) 0.69, Rf (acid) 0.87.

The hydrolysate, from which the aglycone was removed, on usual working up and chromatographic examination in two solvent systems showed the presence of glucose and rhamnose as carbohydrate moities.

The glycoside on methylation with methyl sulphate followed by hydrolysis gave a straw coloured solid which on crystallisation from ethanol separated into straw coloured needles m.p.149-50°. It was characterised as 3-hydroxy-4',5,7-trimethoxy flavone as it showed no depression in melting point on admixture with an authentic sample[247]. The above experiment established the

attachment of carbohydrate moiety as disaccharide in 3-position of the kaempferol glycoside. The quantitative estimation of sugars indicated the presence of two moles of sugars per mole of aglycone. The various steps involved in hydrolysis, location of the sugar position and micro-degradation are shown below:



3,4-Dihydroxydeoxybenzoin

The nuclear oxidation of flavones and related compounds using alkaline persulphate solution is a widely used process. Though the yield is not good, the simplicity of the method more than provides compensation and is, therefore, considered quite suitable for the

preparation of small amounts of material. The application of this method to 5-hydroxy flavones or flavonols leads to 5,8-dihydroxy derivatives. Later it was observed by Seshadri et al that the higher members of 8-hydroxy flavone are more facile to persulphate oxidation than those of 5-hydroxy flavone. The reason for the difficulty in the oxidation of higher members may be that in these compounds the 5-hydroxy group is not activating adequately the 8-position. It may be mentioned that these substances are sparingly soluble in aqueous alkali because of the chelate bond between the 5-hydroxyl and the carbonyl group.

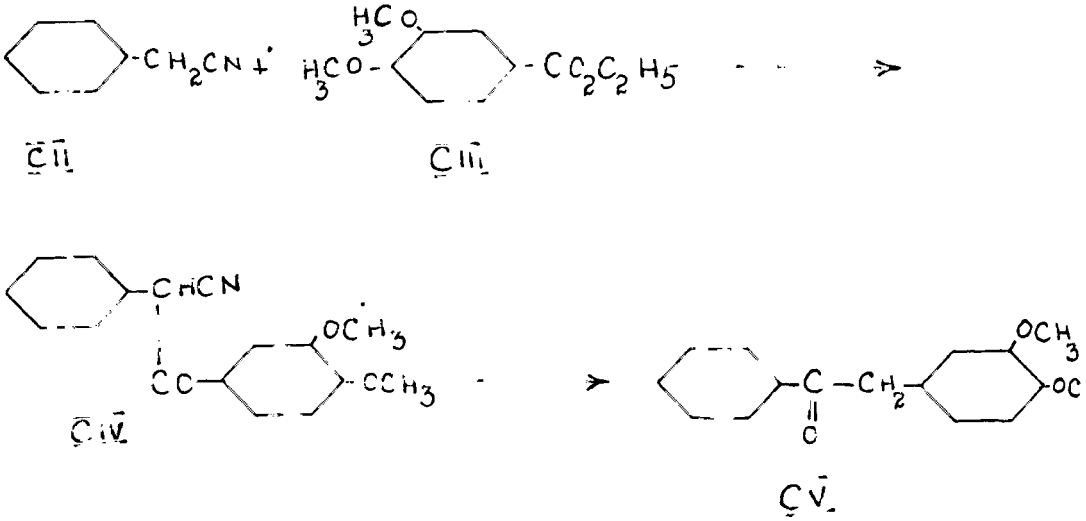
In case of isoflavones the method of nuclear oxidation has been used to a very limited extent. It was, therefore, thought desirable to study the process systematically in the series. To start with, the simplest example of 8-hydroxy isoflavone appeared of interest. A review of the literature revealed that neither the desired isoflavone nor the corresponding deoxybenzoin were reported. The attention was, therefore, diverted towards the synthesis of 2,3-dihydroxydeoxybenzoin, an indispensible starting material for the synthesis of 8-hydroxyisoflavone.

Nenki's and Friedel-Crafts acylation reactions[248-50] are reported to furnish 3,4-dihydroxydeoxybenzoin and its dimethyl ether. No mention of the yields has been made except in one case, where it is stated to be 23%.[249]. The formation of the o-isomer (2,3-dihydroxydeoxybenzoin or its dimethyl ether) is not indicated in either of the reactions. The present work was undertaken with a view to (i) improving the yield of 3,4-dihydroxydeoxybenzoin (ii) exploring the formation of o-isomer along with the para one and (iii) finding out a suitable method for the synthesis of 2,3-dihydroxydeoxybenzoin.

Keeping in view the poor yield in previous methods and the limitations of Hoesch condensation with pyrocatechol nucleus the acylation was carried out in presence of boronfluoride gas. A solution of phenyl acetic acid in chloroform was saturated with boronfluoride gas, pyrocathechol was added and the steam of boronfluoride gas passed again till saturation. The reaction mixture on usual work up gave 3,4-dihydroxydeoxybenzoin in yield of 36%. The substitution of veratrol for pyrocatechol in the reaction gave 3,4-dimethoxydeoxybenzoin in 65% yield. The low yield of 3,4-dihydroxydeoxybenzoin compared to that of its dimethyl ether seems to be due to the formation of a side product which is

receiving attention. 2,3-Dihydroxydeoxybenzoin or its dimethyl ether could not be isolated even in traces.

The Claisen acylation has also been used for the synthesis of 3,4-dimethoxydeoxybenzoin. Phenylacetonitrile CII on acylation with ethylveratrate CIII in presence of sodium ethoxide furnished the intermediate β-ketonitrile CIV.



The ketonitrile CIV on hydrolysis followed by decarboxylation of the β-keto acid furnished the deoxybenzoin CV as colourless needles (benzene-petrol) m.p.83-88° in 60% yield.

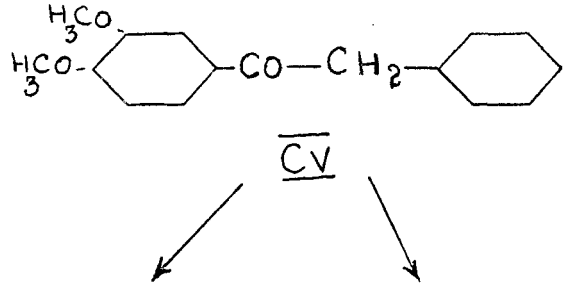
Reis method[224] for the preparation of aliphatic ketones with the improvements made by Jenkins[223] was also

successfully applied for obtaining 3,4-dimethoxydeoxybenzoin. Veratramide (1 mole) was gradually added to an excess (4 moles) of benzyl magnesium chloride in ether and refluxed for 48 hours with occasional stirring. The Grignard complex so obtained on usual work up gave pure 3,4-dimethoxydeoxybenzoin in 71% yield. No evidence of the formation of any abnormal product was obtained in this work. The dimethyl ether on demethylation with hydrobromic and acetic acids gave 3,4-dihydroxydeoxybenzoin in 70% yields.

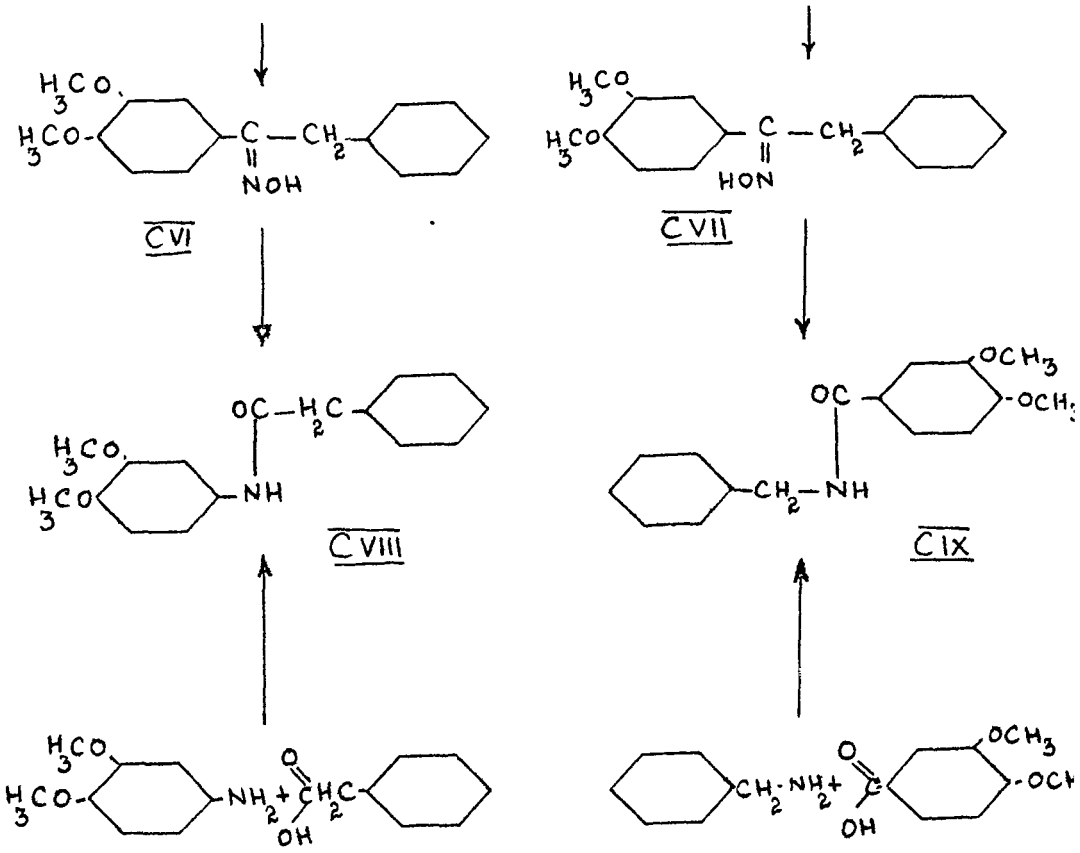
The identity of the 3,4-dihydroxydeoxybenzoin was confirmed by its melting and mixed melting points with an authentic sample synthesised according to the method of Finzi and Lespegnol et al[248-250] and also by the preparation of its 2,4-dinitrophenylhydrazone m.p.243°. 3,4-Dimethoxydeoxybenzoin on demethylation gave a product which on admixture with 3,4-dihydroxydeoxybenzoin showed no depression in the melting point. Further confirmation to the identity of dimethoxydeoxybenzoin was made by the preparation of its oxime m.p.128-29° (Lit.[250] 128-29°) and phenylhydrazone m.p.137-38° (Cf.Lit.[251] m.p.153-54°). The melting point of the phenylhydrazone even after repeated crystallisations could not be raised.

The dimethoxydeoxybenzoin readily yielded a 2,4-dinitrophenylhydrazone m.p.198-99° and a semicarbazone m.p.189-90°. The selenium dioxide oxidation of 3,4-dimethoxydeoxybenzoin gave 3,4-dimethoxybenzil m.p.114.5-115°. The diketone yielded a 2,4-dinitrophenylhydrazone m.p.222-224°.

The identification of 3,4-dimethoxydeoxydeoxybenzoin was also supplemented by a study of the Beckmann-rearrangement of its oxime which may be either CVI or CVII. To determine the configuration of the oxime it was subjected to Beckmann rearrangement with phosphorous pentachloride in dry ether. This gave an anilide which may have the structure CVIII of CIX. As both the anilides were unknown they were synthesised from 4-aminoveratrol[252] and phenyl acetic acid in one case and benzyl amine and veratric acid in the other case. On a comparison of the Beckmann rearranged anilide it was found to be identical with phenylacet-3,4-dimethoxy anilide CVIII, thereby establishing the configuration of the oxime as syn-benzyl type. The various steps involved are schematically shown below:



CV



CVI CVII CVIII CIX

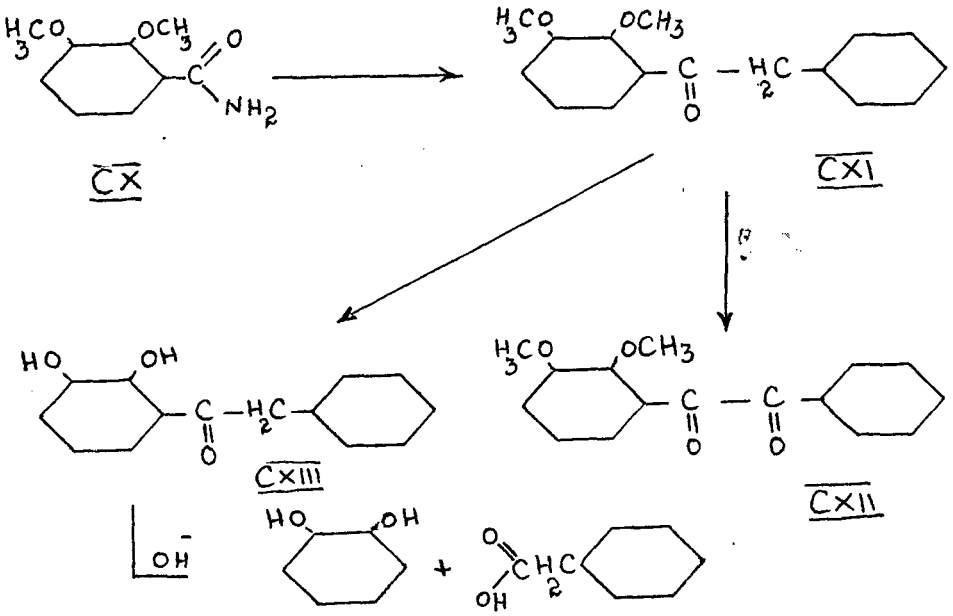
2,3-Dihydroxydeoxybenzoin

The dimethyl ether of the deoxybenzoin CXIII was successfully synthesised by Bei's method for the preparation of aliphatic ketones with Jenkins modification in 76% yield. o-Veratramide CX (1 mole) was gradually added to an excess (4 moles) of benzyl magnesium chloride in ether and refluxed with occasional stirring for 48 hours. The Grignard complex on being worked up in the usual manner gave a brown viscous oil. The brown oil, on

fractionation under reduced pressure (3 mm), gave a colourless fragrant oil, b.p.126-28°, which later solidified in shining plates m.p.54° (yield 12%). The second fraction of the oil (major quantity) was collected at 170-73° as a thick oil of light yellow colour. This on redistillation gave a faint yellow oil b.p.170-73° (3 mm), which readily yielded a 2,4-dinitrophenylhydrazone m.p.180-82° and a semicarbazone m.p.185-86°. The selenium dioxide oxidation of the oil in acetic anhydride gave a new α-diketone (2,3-dimethoxybenzil) CXII in shining light yellow needles m.p.74°. The diketone readily gave a 2,4-dinitrophenylhydrazone m.p.162-64°. The oil on demethylation with hydrobromic and acetic acids gave the corresponding dihydroxydeoxybenzoin CXIII. The dihydroxydeoxybenzoin on crystallisation from benzene petrol gave light yellow aggregates of shining needles m.p.79-81°. It was converted into 2,4-dinitrophenylhydrazone m.p.221-22°.

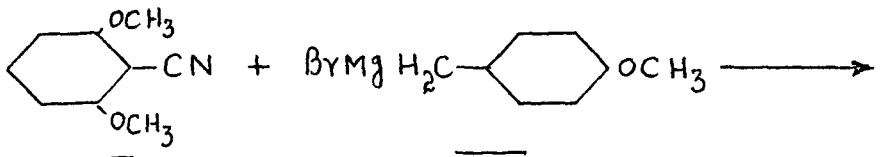
The constitution of the demethylated product as 2,3-dihydroxydeoxybenzoin CXIII was established by its alkaline degradation to pyrocatechol and phenyl acetic acid. Pyrocatechol was identified by co-chromatography with an authentic sample. The acid component was characterised by its melting and mixed melting points with

phenyl acetic acid. The various steps of the reaction are shown below:

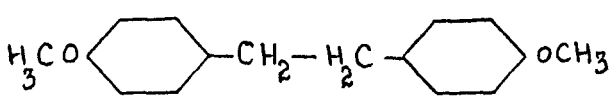


The first fraction which readily solidified and had m.p.54° was characterised as dibenzyl by a mixed melting point with an authentic sample prepared by Clemmensen's reduction of benzil. The formation of dibenzyl in appreciable amount (12%) has, thus, been reported in the course of the preparation of 2,3-dimethoxy-deoxybenzoin. But so far as known to the authors, no earlier mention of such a product is recorded in the preparation of deoxybenzoins from benzyl magnesium chloride and substituted benzamides. The formation of dibenzyl may be a case of <u>preformation</u> as observed in

diallyl formation during the preparation of allyl magnesium bromide. The possiblity of preformation was ruled out by the non-formation of dibenzyl in a blank experiment and a number of reactions where benzyl magnesium chloride and substituted benzamide were used. The formation of dibenzyl in the above case may possibly be explained by the steric or certain other effects coming into play at the time of interaction between benzyl magnesium chloride and substituted amide (2,3-dimethoxybenzamide). These effects tend to suppress the normal reaction and promote the side reaction by a free radical mechanism. Thus relatively unreactive resonance stabilized free benzyl radical may be expected to undergo irreversible dimerisation to dibenzyl. The above arguments find support from a recent observation of Whalley[214] who, in an attempt to prepare 2,4,6-trihydroxydeoxybenzoin from 2,6-dimethoxybenzonitrile CXIV and p-methoxybenzylmagnesiumbromide CXV, obtained di-(p-methoxyphenyl)-ethane CXVI, as the only product of the reaction. The illustration may be taken as an extreme case of suppression of the normal reaction.



CXIV CXV



CXVI

The preparation of 2,3-dimethoxydeoxybenzoin was also carried out by making use of organo zinc compound, when the deoxybenzoin was obtained in 34% yield.

o-Hydroxydeoxybenzoin

Venkataraman et al[253] have recorded the preparation of o-hydroxydeoxybenzoin, in an unstated yield, by Fries rearrangement of phenyl phenylacetate. As the rearrangement yielded both the ortho- and para-derivatives, they were separated by shaking their ethereal solution with 0.5% of sodium hydroxide solution. The ortho derivative crystallised from light petroleum in large colourless hexagonal plates m.p.60° and the para isomer from dilute alcohol in long colourless needles m.p.151°.

The separation of the isomers in our hands was more effective by distilling the reaction mixture under reduced pressure and exhausting each fraction with ligroin. The ligroin soluble fraction on concentration and leaving overnight gave o-hydroxydeoxybenzoin in hexagonal plates m.p.60° and the residue crystallised from dilute alcohol in colourless needles m.p.151°. The yield of the ortho derivative was found to be 24%.

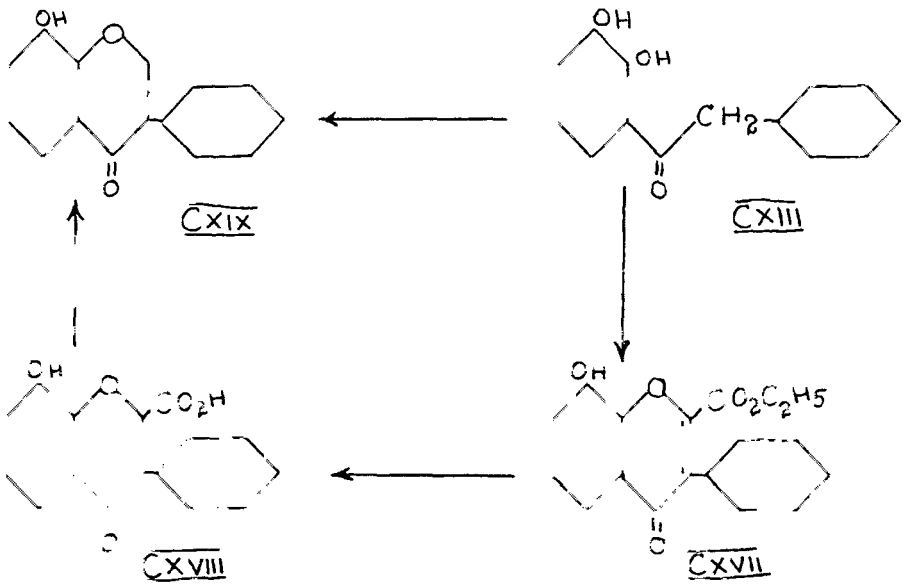
The exclusive formation of o-methoxydeoxybenzoin has now been accomplished by the interaction of benzyl magnesium chloride on o-methoxybenzamide. The oily product b.p.198-200 (4 mm) presumably o-methoxydeoxybenzoin has been obtained in 62% yield. The formation of dibenzyl as a side product (5% yield) has also been noted. The oily product gave a 2,4-dinitrophenylhydrazone m.p.162-64° and a semicarbazone m.p.210°.

The product was demethylated with a mixture of hydrobromic and acetic acids yielding a product which on crystallisation from light petroleum ether melted at 60°. It showed no depression in melting point on admixture with a sample obtained earlier by the Fries rearrangement. It was, therefore, characterised as o-hydroxydeoxybenzoin. It gave a 2,4-dinitrophenylhydrazone m.p.217-19°, a semicarbazone m.p.200° and an oxime m.p.124°.

It is apparent that o-methoxydeoxybenzoin which is recorded here for the first time in 62% yield and gives o-hydroxydeoxybenzoin on demethylation may be considered as a more convenient and useful method for the preparation of the latter compound.

8-Hydroxy Isoflavone

The usual Claisen condensation of 2,3-dihydroxy-deoxybenzoin CXIII with ethyl formate in presence of pulverised sodium gave on subsequent work up 8-hydroxy-isoflavone CXIX m.p.222-24°. It gave a positive sodium amalgam test but strangely enough no ferric reaction. The hydroxyisoflavone dissolved in aqueous sodium carbonate and sodium hydroxide solutions giving a yellow colouration. It gave an acetate m.p.160° and a methyl ether m.p.163°C. The same isoflavone was obtained by the ethoxalylation of 2,3-dihydroxydeoxybenzoin CXIII. 2-Carbethoxy-8-hydroxy isoflavone CXVII m.p.213-15° gave on hydrolysis 2-carboxy-8-hydroxyisoflavone CXVIII m.p.261-63°. The decarboxylation of the latter furnished 8-hydroxyisoflavone CXIX m.p.222-24°. Its melting point with the sample obtained earlier was found undepressed. The steps involve in the reactions are shown below.



No intermediate isoflavanone formation was observed with either of the condensing agents in this case. The structure of the isoflavone CXIX has been proved by mild alkaline hydrolysis. The product of degradation has been characterised as 2,3-dihydroxydeoxybenzoin by co-chromatography with an authentic sample obtained earlier. The spots were revealed by spraying with diazotized p-nitroaniline and ferric chloride solutions. The alkaline degradation of 2,3-dihydroxydeoxybenzoin to pyrocatechol and phenyl acetic acid has also been described.

C_O_N_C_L_U_S_I_O_N_S

C O N C L U S I O N S

The present work described in the thesis consists of two parts:

(A) Anthocyanthin glycosides from flowers of Argemone mexicana Linn., and Ervatamia coronaria Stapf.

(B) Synthetic experiments in Benzopyrone series.

A. I. The bright yellow flowers of Argemone mexicana are found to contain a free aglycone and two new glycosides.

II. The free glycone m.p.304-306° has been characterised as 3'-methoxy-4',5,7-trihydroxyflavonol (isorhamnetin).

III. Both the glycosides carry the same sugar (glucose) and the same aglycone (isorhamnetin).

IV. The glycoside m.p.165-67° has been characterised as isorhamnetin-3-glucoside.

V. The glycoside m.p.208-10° has been characterised as isorhamnetin-7-diglucoside.

VI. The white flowers of Ervatamia coronaria have been found to contain only one glycoside m.p.222-24°. The glycoside has been identified as kaempferol-3-rhamno-glucoside.

B. I. The following methods have for the first time been used for the synthesis of 3,4-dihydroxy-dimethoxydeoxybenzoins in considerable improved yields.

(i) Boronfluoride method

(ii) Grignard method (71% yield)

(iii) Claisen acylation (63% yield)

II. A new α-diketone m.p.114.5-115° (3,4-dimethoxy-benzil) has been obtained by the selenium dioxide oxidation of 3,4-dimethoxydeoxybenzoin.

III. The structure of the methoxydeoxybenzoin has been supported by Beckmann rearrangement of its ketoxime. The configuration of the ketoxime has been established as syn-benzyl type.

IV. Two new compounds, phenyl acet-3,4-dimethoxy anilide m.p.151-52° and N-benzyl (3,4-dimethoxy benzamide) m.p.134-36° have been synthesised for comparison with the Beckmann rearranged product.

V. A new deoxybenzoin (2,3-dimethoxydeoxybenzoin) b.p.170-72° (3 mm) has been obtained in 76% yield by Grignard method.

VI. The organozinc compound has also been used for the synthesis of the deoxybenzoin (yield 34%).

VII. A side product m.p.54° characterised as dibenzyl has been obtained in appreciable amount (12%) during the synthesis of the deoxybenzoin by Grignard method. The formation of dibenzyl may presumably be the result of dimerisation of free-benzyl radical.

VIII. An α-diketone m.p.74° (2,3-dimethoxybenzil) has been obtained by selenium dioxide oxidation of the deoxybenzoin.

IX. The structure of the deoxybenzoin has been established by alkaline degradation.

X. 8-Hydroxy isoflavone m.p.222-24° (not hitherto reported) has been obtained by ethylformate-sodium synthesis and ethoxalylation of 2,3-dihydroxydeoxybenzoin.

XI. The structure of the isoflavone has been proved by alkaline hydrolysis.

XII. Grignard reaction has also been used for the synthesis of o-methoxydeoxybenzoin in 62% yield.

E_X_P_E_R_I_M_E_N_T_A_L

ARGEMONE MEXICANA

Extraction: Fresh flowers of Argemone Mexicana (500 gms) were soaked in petrol ether (40-60) and refluxed for 12 hours. The extract was decanted off and then treated with a fresh quantity of petroleum ether and refluxed again for 12 hours. The extract was again decanted off and the petals were, then, washed with a fresh quantity of petrol. They were completely dried by blowing hot air and exhausted with boiling alcohol till the extract was almost colourless. During the course of extraction a small amount of calcium carbonate was added to avoid the possibility of hydrolysis of the glycoside by free acids, if present in the petals. The combined alcoholic extracts were concentrated under diminished pressure whereby a highly viscous dark brown concentrate was left behind. The concentrate was taken into water (200 cc), and extracted thoroughly with petroleum ether and chloroform to ensure the complete removal of essential oils, fatty, waxy and resinous matters. The petroleum ether and chloroform extracts, on examination showed the absence of flavonoids.

The water extract was then shaken by n-butyl alcohol several times till the butyl alcohol extract was

almost colourless. The water extract was concentrated under diminished pressure on a waterbath, and on examination showed the absence of yellow colouring matter. During the course of extraction of the aqueous solution with n-butyl alcohol and leaving overnight a yellowish brown solid separated which was filtered off and was marked "A". The filtrate on complete recovery of butyl alcohol under reduced pressure on a water bath left a powdery residue of reddish brown colour.

Lead acetate treatment:

The reddish brown powder was dissolved in hot water and allowed to attain the room temperature. The turbid solution on filtration gave a clear filtrate of reddish brown colour. The hot filtrate on treatment with a few cc of 10% lead acetate solution (neutral) resulted in the separation of a small amount of dirty brown greasy precipitate which was femoved by filtration. The precipitate was discarded as it gave no definite product on being worked out.

To the hot filtrate was added more of lead acetate solution (neutral) gradually and with shaking till no more precipitate separated. The bulky yellow precipitate obtained was filtered and labelled "B". The

filtrate was further treated with lead acetate solution followed by ammonia, an orange yellow precipitate was obtained which was filtered and marked "C". The excess of lead acetate present in the filtrate was decomposed by passing hydrogen sulphide gas. The black precipitate was filtered and the filtrate on concentration to a small volume under diminished pressure gave negative tests with Mg + HCl and sodium amalgam, indicating thereby the absence of flavonoids in the filtrate.

Decomposition of the lead salt "B".

The yellow lead salt "B" while still wet was suspended in a large volume of ethyl alcohol, warmed to a temperature of 40-50 and decomposed with hydrogen sulphide gas. It was filtered, the precipitate once more suspended in warm alcohol and hydrogen sulphide gas passed to ensure the complete decomposition of the lead salt. After the filtration the alcoholic extracts were combined together and a current of carbondioxide was passed into the solution to expell most of the H_2S. It was evaporated to dryness under reduced pressure which resulted in leaving behind a yellow solid.

Chromatographic examination of the solid from "B":

The alcoholic solution of the solid obtained above was subjected to chromatographic analysis using Whatman No.1 filter paper and butanol:acetic acid:water (60:10:20), acetic acid:water (60:40) as solvent mixtures and employing both the ascending and descending techniques. The chromatograms were run for twelve hours. After drying at room temperature, the chromatograms on examination under U.V. light revealed four spots, two minor and two major. The minor spots were found to be non-flavonoid.

Chromatographic separation of flavonoids from "B":

Anhydrous acetone (600 cc) was added to magnesium trisilicate (Magnesol) (150 gms) and the mixture was stirred to give a thin slurry. The slurry was added at once to a column 18 mm in diameter, and the sides of the column were rinsed down with dry acetone (200 cc). When the absorbent had settled, leaving a layer of acetone above the surface, an acetone solution containing 500 milligram of the dried substance "B" was added. After the solution had passed into the column, a filter paper circle was placed on top of the adsorbent, and the column was washed thoroughly with acetone. A highly flourescent band, observed under ultraviolet light, was removed during the course of washing of the column. This was found to

be non-flavonoid in nature. The two flavonoid bands (U.V. light) were eluted from the column with ethyl acetate saturated with water. As there was certain overlapping of the bands the separation could not be accomplished effectively. This was further revealed, on chromatographic examination of the two fractions (a & b) on Whatman No.1 filter paper using the solvent butanol:acetic acid:water (60:10:20) and acetic acid:water (60:40), employing both the ascending and descending techniques.

Purification by paper chromatography of the above two fractions (a & b):

The alcoholic solution (10 ml.) of fraction "a" was applied as a streak from a trat pipette in 0.5-1 ml portions to Whatman No.3 filter paper 22 x 18", which had previously been washed with water for 24 hours in a chromatographic cabinet and dried at room temperature. A hand type hair dryer was used to evaporate the spotting solvent. The chromatograms were developed overnight with acetic acid:water (50:40) mixture, and carefully dried in a current of air in a fume cupboard. The positions of the bands were marked under an U.V. lamp. The encircled pigment zones were labelled 'I' and 'II'. The "b" fraction was also purified by chromatographing on Whatman

No.3 papers as in the above case. The bands marked 'I' and 'II' were carefully cut and extracted separately by refluxing them with 70% alcohol. On recovery of the solvent a small amount of residue was left in each case. The two fractions thus separated were tested for homogenity on whatman No.1 paper in butyl alcohol acetic:acid:water (60:10:20) solvent mixture, employing both the ascending and descending techniques. The two fractions on several crystallisation from methanol separately gave minute yellowish brown plates m.p.165-67° and yellow shining needles m.p.304-306°.

Characterisation of the Aglycone m.p.304-6°:

Acetate.

The aglycone was heated under reflux with acetic anhydride (4.5 cc) and fused sodium acetate (300 mg) for two hours. It was poured on crushed ice and left overnight. The solid was collected, washed with water and dried. On crystallisation from ethanol (charcoal) it gave colourless needles (140 mg) m.p.202-204° (Cf.lit.[33] 203-204° for Isorhamnetin tetra acetate)

Anal. Calcd. for $C_{24}H_{20}O_{11}$: C, 59.50; H, 4.16

Found: C, 59.62; H, 4.44

Deacetylation: The above acetate (100 mg) was refluxed with alcohol and hydrochloric acid 1:1 on a water bath for two hours. After adding an equal amount of water, the alcohol was recovered from the yellow solution under reduced pressure. The yellow precipitate was filtered, washed with water and dried. On crystallisation from methanol it gave shining yellow needles (40 mg.) m.p.304-306° (Cf. lit.[34] 306° for Isorhamnetin). The mixed melting point with an authentic sample of Isorhamnetin was found undepressed.

Anal.Calcd. for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82

Found: C, 60.55; H, 3.91

Benzoate:

The aglycone (200 mg) in pyridine (2 cc) was refluxed with freshly distilled benzoyl chloride (15 cc) for half an hour on a sand bath. The mixture while still hot was poured over crushed ice and kept overnight when the oily mass first formed became an amorphous solid. It was treated with an excess of sodium bicarbonate solution, filtered and washed thoroughly with water and dried. On crystallisation from ethyl acetate it gave colourless plates m.p.138-40°.

Methylation: The aglycone (200 mg) in dry acetone (100 cc) was refluxed with methyl sulphate (1 cc) and freshly ignited potassium carbonate (3 g) for 30 hours. It was filtered and the residue washed several times with boiling acetone. On distilling off the solvent, a brown viscous semisolid mass was left behind. It was washed with hot petroleum ether to remove the excess of methyl sulphate. The solid residue on crystallisation from methanol and then with ethyl acetate gave colourless needles, melting and mixed melting point 152° (Cf.lit.[254] 151-52° for pentamethyl quercetin) with an authentic specimen of pentamethyl quercetin.

Anal. Calcd. for $C_{20}H_{20}O_7$:	C, 64.51;	H, 5.41
Found:	C, 64.42;	H, 5.38

Chromatographic, Ultraviolet and Infra-red Spectral evidences.

The co-chromatography of the aglycone with an authentic sample of isorhamnetin on Whatman No.1 paper using butanol:acetic acid:water (60:10:20) and phenol saturated water as solvent systems gave identical spots. The spots were revealed in U.V.light, U.V.light and ammonia vapours, by spraying with solutions of ferric chloride, sodium carbonate, p-nitro aniline and bisdiazotized benzidin.

Rf values: 0.83 (n-butanol:acetic acid:water, 40:10:50), (Cf. lit.[242,254] 0.80, 0.83).

Absorption: λ_{max}^{EtOH} 256 mμ and 373 mμ (Cf. lit.[34,254] λ_{max}^{EtOH} 255 mμ and 375 mμ ; λ_{max}^{EtOH} 256 mμ and 373 mμ).

The infra red spectra of the aglycone and authentic isorhamnetin were found to be superimposable. CO and OH frequencies, were measured in Nujol using a double beam Perkin-Elmer spectrometer Model 137, in Cm^{-1} 1655, 3160 respectively. (Cf. lit.[33] CO and OH frequencies in Cm^{-1} in Nujol 1655, 3160 respectively).

Identification of Isorhamnetin-3-glucoside,(M.P.165·67°):

Glycoside acetate: The crystalline glycoside 300 mg., anhydrous pyridine (4 cc) and acetic anhydride (4 cc) were heated at 85-95° on a water bath for 3 hours. The reaction mixture was cooled and poured over crushed ice. The precipitate filtered, washed and dried. On crystallisation from dibute ethanol it gave colourless needles m.p.135-36°.

Deacetylation: The glycoside aceate (100 mg) was kept at 0°C with 0.1N methanolic sodium methoxide (25 cc).

After 24 hours, it was neutralised with dil. HCl. The precipitated solid was filtered and dried. On several crystallisations from methanol it gave minute yellowish brown plates m.p.165-67°.

Anal.Calcd. for $C_{22}H_{22}O_{12},2H_2O$: C, 51.36; H, 4.67
Found: C, 51.5; H, 4.74

Hydrolysis: The anhydrous glycoside (500 mg) was hydrolysed by refluxing with 125 cc of 0.6 N hydrochloric acid. The hydrolysis appeared to be completed within a few minutes. The refluxing was continued for two hours to ensure complete hydrolysis. After leaving overnight, the yellow aglycone thus separated out was filtered, washed well with water and dried in an oven at 120°. The crude product crystallised from methanol in yellow needles m.p.304-306° (Cf. lit[34]. 306 for isorhamnetin). The aglycone showed no depression in melting point on admixture with an authentic sample of isorhamnetin. Its identity as isorhamnetin was further confirmed by co-chromatography, ultraviolet, infra-red absorption spectra and colour reactions as given earlier.

Anal.calcd. for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82
Found: C, 60.88; H, 3.80

It gave an acetate m.p.202-204° and a pentamethyl ether of quercetin m.p.151-52° (Cf.lit.[254]. 151-52°).

Chromatographic identification of sugars:

The acidic filtrate left after filtering the aglycone was extracted with ether and then with ethyl acetate to ensure the complete removal of any residual aglycone. The solution was heated for 2-3 minutes with a pinch of charcoal at 40-50° on a water bath to remove the colouring and inorganic matter. The clear filtrate obtained was concentrated to a syrup in vacuum over NaOH pellets. The concentration was continued till the syrup was neutral to litmus paper. The syrup was chromatographed on Whatman No.1 filter paper using butanol:acetic acid:water (40:10:50) and n-butanol:water:ethanol (60:28.5:16.5) as solvent mixtures, employing the descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying at room temperature in fume cupboard, were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-5° showed the presence of glucose only. The osazone of the sugar was also prepared from the concentrate by the usual method, which was separated in hot within 4 to 5 minutes; it melted at 204-205° showed no depression in melting point when mixed with the authentic specimen of glucosazone.

Estimation of sugar:

The anhydrous glycoside (45.2 mg) was hydrolysed by refluxing for two hours with 2% H_2SO_4. After cooling overnight, the aglycone was filtered, washed, dried and weighed (29.6 mg). Thus the ratio of the aglycone to the glycoside is 65.4% and this ratio indicates the presence of one mole of sugar per mole of aglycone.

The quantitative estimation of sugar by Smogyi's copper micro method gave the value (.44 cc) which corresponds to 1 mole of sugar per mole of aglycone.

Location of the Sugar position of the glucoside m.p.165-67°:

Glucoside (300 mg) was dissolved in dry acetone and was refluxed with an excess of dimethyl sulphate (1.2 cc) and ignited potassium carbonate (4 gms) for 36 hours on a water bath. The mixture was filtered and the residue was washed with hot dry acetone. After distilling off the solvent from the filtrate a reddish brown oily residue was left behind. The excess of dimethyl sulphate was removed by washing the methylated product several times with hot petroleum ether. Repeated attempts to crystallise the semisolid mass proved fruitless. It was, therefore, directly hydrolysed by refluxing with 7% H_2SO_4

for two hours. The reaction mixture was left over-night when a faintly yellowish powder was separated out. It was filtered washed with water and dried. On several crystallisations from ethanol it gave straw coloured needles melting at 193°. (Cf.lit.[34] 192-94° for 3',4',5,7-tetramethyl quercetin). A mixture of this ether with 3',4', 5,7,-tetramethyl quercetin showed no depression in melting point.

Anal.Calcd.for $C_{19}H_{18}O_7$	C, 63.68;	H, 5.02
Found:	C, 63.60;	H, 4.88

On acetylation it gave a product which on crystallisation from dilute methanol gave shining colourless needles m.p.160-61° (Cf.lit.[34] 160° for 3 acetoxy 3',4',5,7,-tetramethyl quercetin).

Decomposition of the lead salt "C":

The orange yellow lead salt "C" while still wet was ground thoroughly and suspended in excess ethyl alcohol, warmed to a temperature of 40-50° and was deleaded by passing hydrogen sulphide gas. It was filtered and the precipitate was washed by hot alcohol. The filtrate and the washings were combined together and evaporated to dryness under reduced pressure. A semi-solid

mass was obtained. Chromatographic examination on Whatman No.1 paper using butanol:acetic acid:water (60:10:20) and acetic acid:water (60:40), employing both the ascending and descending techniques, revealed the presence of one non-flourescent (U.V. Light) spot with a little traling.

Purification of the glycoside, from "C":

A 500 mg. sample from "C" was dissolved in dry acetone (50 cc) and the solution was then passed through a 60 mm column packed to a depth of 160 mm with magnesium trisilicate. After the solution has passed into the column, a filter paper circle was placed on top of the absorbent, and water saturated ethyl acetate was carefully added. Elution with ethyl acetate solution showed a single yellow band, (visible light) and two bands one highly flourescent and the other non-flourescent (U.V. light). The bands were several centimeters apart and were easily collected as two fractions. Chromatographic examination of two fractions on Whatman No.1 filter paper using the solvent butanol:acetic acid:water (60:10:20) and acetic acid:water (60:40), employing both the ascending and descending techniques, revealed the homogenity of each of the fraction (U.V.light). The flourescent part gave negative tests with Mg + HCl, and sodium amalgam. No

colour was developed on exposing the chromatogram to ammonia vapours. On several crystallisations from methanol it gave shining colourless plates m.p.124°.

The non-flourescent part on crystallisation from methanol gave yellowish brown plates m.p.165-67°. It gave a salman pink colouration with Mg + HCl and also on reduction with sodium amalgam. It gave a dark red colouration with Zn + HCl and a bright yellow colour with Wilson boric acid reagent. It showed no depression in melting poin on admixture with a sample of isorhamnetin 3-glucoside, previously obtained from the fraction "B". The co-chromatography of the two glycosides on Whatman No.1 filter paper using butanol:acetic acid:water (40:10:50) as solvent system gave identical spots.

On hydrolysis the glycoside gave an aglycone m.p.304-306°. It was characterised as isorhamnetin, as described earlier. The chromatographic examination of the filtrate showed the presence of only glucose. Position of the sugar was determined by usual methods of methylation followed by hydrolysis, whereupon straw coloured needles m.p.193° (Cf.lit.[34] 191-92°) were obtained.

Identification of Isorhamnetin-7-diglucoside:

The yellow solid "A" which separated on leaving the n-butanol extract overnight was dissolved in a large excess of boiling methanol. On cooling the solution the yellow microscopic needles m.p.202-205° separated. On several crystallisations from methanol (large excess) the melting point rose to 208-10°.

Chromatographic examination in butanol:acetic acid:water (60:10:20) and acetic acid:water (60:40) showed the homogenity of the glycoside.

Acetate: Anhydrous glycoside (300 mg), pyridine (4 cc) and acetic anhydride (4 cc) were heated on a water bath at 85-95° for three hours. On usual work up and crystallisation from methanol it gave colourless needles m.p.148-50°.

Deacetylation: The glycoside acetate (100 mg) was kept in 0.1 N methanolic sodium methoxide (25 cc) at 0° for 24 hours. The product was worked out as described earlier. On several crystallisations from methanol it gave yellow needles m.p.208-10°.

Anal.Calcd. for $C_{28}H_{32}O_{17}$: C, 52.5; H, 5.00

Found: C, 52.7; H, 4.98

It gave a salmon pink colouration with Mg + HCl and also on acidification after reduction with sodium amalgam. It gave a bright yellow colour with Wilson boric acid reagent.

Hydrolysis of the glycoside:

The anhydrous glycoside (500 mg) was hydrolysed by refluxing with 125 cc of 0.6 N hydrochloric acid, for two hours. After leaving over-night, the aglycone was filtered off, washed and dried. On crystallisation from methanol it gave yellowish brown plates m.p.304-306° (Cf.lit.[34] 306 for isorhamnetin). The aglycone showed no depression in melting point on admixture with an authentic sample of isorhamnetin. Its identity as isorhamnetin was further confirmed by co-chromatography, ultraviolet and infra-red spectra as given earlier.

Anal.Calcd. for $C_{16}H_{12}O_7$:	C, 60.76;	H, 3.82
Found:	C, 60.68;	H, 3.80

It gave an acetate m.p.202-204° (Cf.lit.[33] 203-204°). The chromatographic examination of the filtrate showed the presence of only glucose. This was confirmed by the formation of osazone m.p.204-206°.

Estimation of sugars: The anhydrous glucoside (46.1 mg) was hydrolysed by refluxing for two hours with 2% H_2SO_4. After cooling overnight, the aglycone was filtered, washed, dried and weighed (20.6 mg.). Thus the ratio of the aglycone to the glycoside is 44.7% and this ratio indicates the presence of two moles of sugar per mole of aglycone.

Somogyi's copper micro method gave the value (1.64 cc) which also corresponds to two moles of sugar per mole of aglycone.

Location of the sugar position of the glycoside:

The methyl ether of the glycoside was prepared by refluxing a mixture of glucoside (300 mg), acetone (100 cc), methyl sulphate (1.5 cc) and freshly ignited potassium carbonate (4 g) on a water bath until a few drops of the mixture showed no more colouration with ferric chloride. The usual work up of the reaction mixture followed by hydrolysis gave a solid product of light brown colour. On several crystallisations from dilute ethanol it gave straw coloured needles m.p.284-85° (Cf.lit.[255] 284°). This on admixture with an authentic specimen of 3',4',3,5,-tetra-methyl quercetin showed no depression in melting point.

Anal.Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.02

Found: C, 63.59; H, 4.91

It gave an acetate m.p.174° (Cf.lit.[255] 174 for 7 acetoxy 3',4',3,5,tetramethyl quercetin).

Ervatamia Coronaria

Extraction: Fresh white flowers (4 kg.) of Ervatamia Coronaria, collected on the campus of the Faculty of Science, Muslim University, Aligarh, were dried in air. The air dried flowers were extracted twice with petroleum ether (40-60°). The extract was decanted off and the petals were completely dried by blowing hot air through them. The dry flowers were then exhausted with ethanol. During the course of refluxing with ethanol a pinch of calcium carbonate was added to avoid the possibility of hydrolysis of glycosides by free acids, if present in the petals. The combined alcoholic extracts were concentrated under diminished pressure whereby a highly viscous dark-brown concentrate was left behind. It was taken in hot water (300 cc); cooled and filtered. The residue was non-flavonoid in nature. The filtrate was extracted thoroughly with petroleum ether and chloroform respectively to ensure complete removal of essential oils, fatty, waxy and

Spelling Stereochemistry.

Hydriodic acid.

Things to remember: "Filtered" means to be passed through a filter. Precipitate do not pass through the filter.

Far too often you omit "was". For example, on page 126 line 18 you write "The precipitate filtered, and washed several times with water." This means the precipitate did the filtering and the washing whereas, in fact, you did the filtering and washing.

resinous matter. The aqueous extract was then shaken with n-butyl alcohol several times. The water extract was concentrated to a small volume under diminished pressure on a water bath, and when examined showed the absence of yellow colouring matter. The combined n-butyl extracts, on recovery of the solvent, left behind a reddish brown sticky mass.

Lead acetate treatment: The reddish brown sticky mass was dissolved in hot water and the insoluble material filtered off. The hot filtrate on treatment with a few cc of lead acetate solution (neutral) resulted in the separation of a small amount of dirty brown greasy precipitate which was removed by filtration. It was discarded as it gave no definite product on being worked out. The filtrate was then treated with lead acetate solution (neutral) but no precipitate appeared. A bulky orange yellow precipitate was separated out by the addition of ammonia to the filtrate. The precipitate filtered, and washed several times with water. The filtrate, on deheading, showed no tests for flavonoids.

Decomposition of the lead salt:

The orange yellow salt while still wet was suspended in a large volume of ethanol, warmed to a temperature of 40-50° and treated with hydrogen sulphide gas till

the decomposition was complete. It was filtered and the residue was washed with warm ethanol. The washings and the filtrate were combined and a current of carbondioxide was passed into the solution to expell most of the hydrogen sulphide gas. It was evaporated to dryness, under reduced pressure, on a water bath which resulted in leaving behind a dirty brown non-crystallisable solid.

Chromatographic analysis: The dirty brown amorphous solid was dissolved in alcohol and subjected to chromatographic analysis using Whatman No.1 filter paper and butanol:acetic acid:water (60:10:20), acetic acid:water (60:40), phenol satured with water, as solvent mixtures, employing both the ascending and descending techniques. The chromatograms were dried in air. On examination under U.V. light they revealed a flourescent spot, and three blue spots. Spraying with aqueous sodium carbonate solution and also with alcoholic ferric chloride solution showed only one zone.

Purification of glycoside by column chromatography:

A 500 mg of the glycoside was dissolved in dry acetone (50 cc) and the solution was then passed through a 60 mm column packed to a depth of 160 mm with magnesium trisilicate. The flavonoid was adsorbed at the top of

the column. The column was washed thoroughly with dry acetone and water saturated ethyl acetate was carefully added. Elution with ethyl acetate solution showed a single flourescent band (U.V.light). The ethyl acetate solution was concentrated on a waterbath and left overnight in an ice box. Light brown powdery mass separated, which on several crystallisations from methanol gave light brown needles m.p.222-224° (Cf.lit[256] 223-224° for Kaempferol 3-rhamno-glucoside).

Identification of Kaempferol 3-glycoside (m.p.222-224°)

Glycoside acetate: Anhydrous glycoside (300 mg), anhydrous pyridine (4 cc) and acetic anhydride (4 cc) were heated at 85-98° on a water bath for 3 hours. The reaction mixture was poured over crushed ice. The precipitate was filtered, washed and dried. On crystallisation with dilute ethanol it gave colourless needles m.p.158-60°.

Deacetylation: The glycoside acetate (100 mg) was dissolved in 0.1 N sodium methoxide (25 cc) and kept at 0° C. After 24 hours, it was neutralised with dilute HCl. The precipitated solid was filtered and dried. On crystallisation from methanol it gave light brown needles m.p.222-24°.

Anal.Calcd.for $C_{27}H_{30}O_{15}$: C, 54.37; H, 5.36

Hydrolysis: Anhydrous glycoside was heated with 7% HCl (150 cc) on a water bath. The heating was continued for 3 hours to ensure the complete hydrolysis. The solution was cooled overnight, the aglycone filtered off, washed and dried. The crude product melted at 272-76°. It was crystallised from dilute pyridine in light yellow needles m.p.276-78° (Cf.lit.[256] 276-78° for Kaempferol).

Characterisation of aglycone m.p.276-78°.

Acetylation: Aglycone (150 mg) was heated under reflux with acetic anhydride (4.5 cc) and fused sodium acetate (300 mg) for two hours. The mixture was poured on crushed ice and left overnight. The solid was collected, washed with water and dried. On several crystallisations from ethanol (charcoal) it gave shining colourless needles m.p.180-82° (Cf.lit.[257] 180-82°).

Deacetylation: The above acetate (100 mg.) was refluxed with alcohol and hydrochloric acid (1:1) on a water bath for half an hour. Equal amount of water added, and the alcohol was recovered under diminished pressure on a water bath. The yellow precipitate obtained on filtration was washed with water and dried. On several crystallisations

from dilute pyridine it gave yellow needles m.p.276-78° (Cf.Lit.[256] 276-78° for Kaempferol).

Anal.Calcd.for $C_{15}H_{10}O_6$: C, 62.93 ; H, 3.49

Found: C, 62.86 ; H, 3.12

Chromatographic identification of the aglycone (M.P.276-78°)

The co-chromatography of the aglycone with an authentic sample of Kaempferol on Whatman No.1 filter paper, using butanol:acetic acid:water (60:10:20) acetic acid:water (60:40)/as solvent system and employing both the ascending and descending techniques, gave the identical spots. The spots were revealed in U.V.light, U.V.light and ammonia vapours, by spraying with solutions of ferric chloride, sodium carbonate and bis-diazotized benzidine.

Rf value: 0.86 (n-butanol:acetic acid:water, 60:10:20) (Cf.Lit. 0.85); 0.50 (acetic acid:water, 60:40), (Cf.Lit. 0.50).

Micro degradation of the aglycone:

The aglycone (.5 mg) few drops of water and potassium hydroxide (100 mg) were kept at 240° for 3 minutes. The initial dark orange colour was disappeared, the reaction mixture cooled, diluted with water, acidified and extracted with ether (.5 cc) twice. The ether extract was

run on paper chromatograms, using Whatman No.1 filter paper and employing ascending technique in three solvent system, n-butyl-alcohol:acetic acid:water (60:10:20). The chromatograms were dried at room temperature, the examination of fragments revealed two spots by spraying with bis-diazotized benzidine, indistinguishable from those of authentic samples of phloroglucinol and p-hydroxy benzoic acid, Rf: (phenol) 0.69; Rf: (acid) 0.87.

Chromatographic identification of sugars:

The filtrate from which the aglycone was removed was concentrated to a syrup in vacuum. The concentration was continued till the syrup was neutral to litmus paper. The sugars were identified chromatographically in two solvent systems, n-butanol:acetic acid:water (40:10:50) and n-butanol:water:ethanol (60:28.5:16.5) using authentic sugars as checks. Aniline phthalate and p-anisidine phosphate solutions were used as spray reagents. The Rf. values of the sugars were identical with those of glucose and rhamnose. This analysis confirmed the presence of glucose and rhamnose in the glycoside. (Rf values: 0.18, 0.105 for glucose; 0.37, 0.285 for rhamnose).

Location of the sugars position in the glycoside.(M.P.222-24°):

A suspension of finely powdered glycoside (200 mg) in anhydrous acetone (80 cc) was refluxed with an excess of methyl sulphate (0.8 cc) and ignited potassium carbonate (2.5 gm) for 48 hours with frequent shaking. The mixture was filtered and the residue was washed with hot acetone. After distilling off the solvent from the filtrate a reddish brown oily residue was left behind. The excess of methyl sulphate was removed by washing the methylated product several times with petroleum ether. A light brown solid was obtained, which could not be crystallised. It was directly hydrolysed by refluxing with 7% aqueous sulphuric acid for two hours and the reaction mixture was cooled in an ice bath, when a straw coloured solid separated out. It was filtered, washed and dried. On several crystallisations from ethanol it gave straw coloured needles m.p.149-50° (Cf.Lit.[258] 149-50° for 3-hydroxy-4',5,7-trimethoxy flavone). A mixture of this ether with 3-hydroxy, 4',5,7-trimethoxy flavone showed no depression in melting point.

Anal.Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.87

Found: C, 65.52; H, 4.80

Estimation of sugars: The anhydrous glycoside (39.5 mg) was hydrolysed by refluxing for two hours with 2% H_2SO_4. After cooling overnight the aglycone was filtered, washed, dried and weighed (19.3 mg). Thus the ratio of the aglycone to the glycoside is 48.8% and this ratio indicates the presence of two moles of sugars per mole of aglycone.

2:4-Dihydroxyphenyl-benzyl ketone:Borontrifluoride method:

Phenyl acetic acid (35 gms) was dissolved in chloroform (60 cc) and a stream of borontrifluoride was passed into the solution cooled to 10-15°C. After the separation of phenyl acetic acid-borontrifluoride complex (30 mts.) pyrocatechol (15 gms) was added to it. The colour of the solution immediately turned yellow. The passage of the boron-trifluoride gas was continued for another quarter of an hour, and the reaction mixture was left overnight at room temperature (25-28°). The clear yellow solution was poured into crushed ice and left for two hours. It was extracted with ether. The ether-chloroform layer was separated, washed thoroughly with sodium bicarbonate solution and then with water, and was dried over anhydrous sodium sulphate. The solvent was removed by distillation, when a yellow oil was obtained, which solidified later. The solid was first crystallised with water and thereafter with alcohol-benzene in almost colourless needles m.p.173-174° (Cf.Lit.m.p.173°) yield 11.2 gms; 36% (Cf.Lit.[249] 23%).

Calcd.for $C_{14}H_{12}O_3$:	C, 73.68;	H, 5.26
Found:	C, 73.58;	H, 5.05

The mixed melting point of the dihydrodeoxybenzoin with a sample, prepared according to Finzi (Nencki's Method), showed no depression. It gave a 2:4-dinitrophenyl hydrazone, which on crystallisation from ethyl acetate separated in light orange needles m.p.243°.

Anal.Calcd.for $C_{20}H_{16}C_6H_4$:	N, 13.71
Found:	N, 13.60

Methylation: The deoxybenzoin (2 gms), sodium hydroxide solution (28 cc, 50%), methyl sulphate (12 cc) were heated, in a three necked flask fitted with a stirrer, on a steam bath for two hours. The addition of sodium hydroxide solution and methyl sulphate was done alternately in small lots. The reaction mixture was made strongly alkaline in the end by the addition of 4 cc of sodium hydroxide solution, and the heating was continued for another half an hour. The cooled reaction mixture was extracted with ether and dried over anhydrous magnesium sulphate. On distilling off the ether the yellowish brown oily mass was left behind, which crystallised from benzene-petrol in colourless shining needles m.p.87-88°(Cf.Lit.[250] m.p.88°, 91-93°).

Anal.Calcd.for $C_{16}H_{16}O_3$:	C, 74.97; H, 6.29
Found:	C, 74.75; H, 6.06

3,4-Dimethoxydeoxybenzoin-Claisen acylation.

Powdered and dried ethyl veratrate (63 gm), mixed with benzyl cyanide (23.4gm), was added to the hot solution of sodium ethoxide, prepared from clean sodium (6 gms) and absolute alcohol (70 cc). The mixture was thoroughly shaken and heated on a water bath for four hours. It was left overnight to cool. The sodium salt thus formed was cooled to 10°C and filtered under suction. The residue was washed four times with 25 cc portions of dry ether. The sodium salt, still wet with ether, was dissolved in water (125 cc), at room temperature (28-30°), again cooled to 10°C and glacial acetic acid (9 cc) was added with vigorous stirring, when a colourless precipitate of nitrile was separated. The precipitate was filtered, washed with water and dried, (m.p.180-82°).

3:4-Dimethoxydeoxybenzoin:(Hydrolysis of nitrile):

Concentrated sulphuric (35 cc) was placed in a flask cooled to 0°C. The moist phenyl acetonitrile obtained above was added to it slowly, with shaking, and the temperature was kept below 20°C. After the addition, the flask was warmed on a water bath and water (175 cc) was added rapidly. The mixture was then heated to 140°C for 3 hours with occasional shaking. An oily layer was sepa-

rated. The solution was extracted with ether. The ether layer was washed with water, then with sodium carbonate solution and finally with water and dried over anhydrous magnesium sulphate. The ether was distilled off and the residue left was crystallised by benzene-petrol in colourless needles m.p.86-88 (Cf.Lit.[249,50] 88, 92-93°); yield, 17.6 gms.(63%).

Anal.Calcd.for $C_{16}H_{16}O_3$:	C, 74.97;	H, 6.29
Found:	C, 74.90;	H, 6.1

3:4-Dimethoxydeoxybenzoin:Borontrifluoride method:

Veratrol (15 gms) was added to phenyl acetic acid-borontrifluoride complex, prepared by 35 gms of phenyl acetic acid, and a current of borontrifluoride was passed for 25 minutes. The reaction mixture was, on leaving overnight, turned dark red. It was poured over crushed ice. On subsequent work up as described previously, a brownish oil was obtained. The brown oil on crystallisation from benzene-petrol gave colourless aggregates of silky needles m.p.87-88° (Cf.Lit.[249,250] m.p.88°, 91-93°), yield 18 gms (65%).

Anal.Calcd.for $C_{16}H_{16}O_3$:	C, 74.97;	H, 6.29
Found:	C, 74.87;	H, 6.3

(i) Phenyl hydrazone: A solution of colourless phenyl hydrazine hydrochloride (500 mg) and sodium acetate (800 mg) in water (5 cc) was added to a solution of 3:4-dimethoxy phenylbenzyl ketone (200 mg) in a little alcohol. A little more alcohol was added to clear the solution and refluxed on a water bath for 30 minutes. The reaction mixture was cooled over night in an ice box. The crystalline solid was filtered off, and on several crystallisations from dilute ethanol gave light brown needles m.p.137-38° (Cf.Lit.[251] 153-54).

Anal.Calcd. for $C_{22}H_{22}O_2N_2$: N, 8.09

Found: N, 8.09

(ii) Semi-carbazone: Semicarbazide hydrochloride (500 mg), crystallised sodium acetate (700 mg) were dissolved in water 5 cc). A solution of 3:4-dimethoxydeoxybenzoin (250 mg) in alcohol was added, the mixture was shaken for 2 hours, and left over night. The precipitated solid was

filtered, washed, and dried. On crystallisation from ethanol it gave colourless needles m.p.189-90°.

Anal.Calcd. for $C_{17}H_{19}O_3N$: N, 13.40

Found: N, 13.17

(iii) 2:4-Dinitrophenylhydrazone: To a hot solution of 2:4-dinitrophenylhydrazine (500 mg) in alcohol (8 cc) was added hydrochloric acid (1 cc) and a solution of the deoxybenzoin (250 mg). The reactant mixture was heated just to boiling, and cooled. The precipitated solid filtered, washed with 20% hydrochloric acid followed by water and dried. On crystallisation from ethyl acetate it gave light reddish brown needles m.p.198-99°.

Anal.Calcd. for $C_{22}H_{20}O_6N_4$: N, 12.84

Found: N, 13.27

3:4-Dimethoxydeoxybenzoin (Grignard method):

Magnesium (4 gms) was placed in a dry 3 necked flask (250 cc) fitted with a mercury sealed stirrer, a dropping funnel (50 cc), and a condenser provided at its upper end with a drying tube containing a mixture of $CaCl_2$ and sodalime, and a gas inlet tube reaching to the bottom of the flask. A rapid stream of dry and oxygen

free nitrogen was passed through the apparatus to expell the air. Dry ether (20 cc) and a small x'al of iodine were then added in the flask. The flow of nitrogen was slowed down and about 10 cc of a solution of freshly distilled benzyl chloride (11.4 gms) in dry ether (50 cc) was added. The ether started boiling. The rest of the solution of benzyl chloride was added at a rate that gentle refluxing is mentioned. The mixture was refluxed for about 15 minutes.

With the stirrer still running, finely powdered dry tetramide (3.0 gms) was added in small portions at a time. The rate of addition of amide was determined by the vigour of the reaction. The solution was refluxed in an atmosphere of nitrogen, with occasional stirring, for 48 hours. The flask was placed in an ice bath and then hydrolysed with cold sulphuric acid (10%). After standing at room temperature for an hour the ether was separated. The water layer was heated on the hot plate for half an hour, cooled and extracted with ether. The combined ether extracts were dried over anhydrous magnesium sulphate. The ether was distilled off leaving behind an oily product. The oil on crystallisation from benzene-petrol gave fine silky needles (4 g; 71%) m.p.87-88°. It showed no

depression in melting point on admixture with a sample previously prepared by borontrifluoride.

Anal.Calcd.for $C_{16}H_{16}O_3$: C, 75.97; H, 6.29
Found: C, 76.00; H, 6.3

Demethylation:

3:4-Dimethoxydeoxybenzoin (6.5 gms) was refluxed with hydrobromic acid (Sp.Gr. 1.5; 150 cc) for 5 hours. It was poured in cold water, when it separated as a yellow solid. The yellow solid was filtered off, and washed with water thoroughly. The filtrate was extracted with water and then with sodium bicarbonate solution. On distilling off the ether, a residue was left behind. The two residues were combined and crystallised from water in light brown needles m.p.173-74°(Cf.Lit.[248] m.p.173°)

Preparation of the oxime:

Hydroxyl amine hydrochloride (5 gms), water (5 cc), caustic soda (20 cc; 10%) and 3:4-dimethoxydeoxybenzoin (2 gms) were mixed together and just enough alcohol was added to get a clear solution. The mixture was refluxed for fifteen minutes and left overnight in a refrigerator. An oily product was separated, which on

several crystallisations from dilute alcohol gave the oxime in shining colourless needles m.p.128-29° (Cf.Lit.[250] 128-129°).

Anal.Calcd.for $C_{16}H_{17}O_3N$: N, 5.16

Found: N, 5.15

3:4-Dimethoxyphenyl-benzyl amine:

A mixture of benzyl amine (1 g.) and veratric acid (1 g.) was heated at 180-190° for three hours. The resulting product was triturated first with dilute sodium hydroxide solution and then with dilute hydrochloric acid and finally washed with water. The crude mass on crystallisation from benzene petrol gave colourless needles m.p.134-35°.

Anal.Calcd.for $C_{16}H_{17}O_3N$: N, 5.17

Found: N, 5.21

4-Amino-Veratrol (3:4-dimethoxy aniline):

An alkaline solution of sodium hydpochlorite was prepared by passing chlorine into a mixture of cracked ice (30 gms) and a cold solution of NaOH (8 gms in 50 cc). Veratric amide (5.8 g.) obtained from veratroyl chloride

and concentrated ammonia, was added in one portion to alkaline solution of sodium hypochlorite, and the mixture was warmed slowly on a water bath, with mechanical stirring. The material immediately darkened in colour and at 50-55° (internal temperature) oily droplets started separating. The temperature was raised gradually to 70° and maintained at this temperature for one hour. A solution of NaOH (12 cc, 30%) was added slowly and the temperature was raised to 80° for an additional hour. On cooling the reactant mixture 4-aminoveratrol separated as a red crystalline mass. The crude amine was filtered with suction, washed thoroughly with ice-cold water and pressed. It was distilled under reduced pressure at 172-174°/24 mm. The distillate (3.9 gms) solidified quickly to colourless crystalline mass m.p.87.5-88°.

Phenylacet-3,4-dimethoxy anilide:

The anilide was obtained from 4-amino veratrol (1 g.) and phenyl acetic acid (1 g) and proceeding in the manner described under the preparation of 3,4-dimethoxy-phenyl benzyl amine. The anilide was crystallised from ethanol in colourless needles m.p.151-52°.

Anal.Calcd. for $C_{16}H_{17}O_3N$: N, 5.17

Found: N, 5.27

Beckmann rearrangement of the oxime:

Oxime (1 gm) was dissolved in dry ether (30 cc). Phosphorous pentachloride (1.5 gm) was added portionwise to the mixture. The solution was shaken and kept cooled during the addition. The reaction mixture was left at room temperature for half an hour, the mixture was poured into ice cold water (100 cc) and ether drived off by means of a stream of air. The crude anilide separated, was filtered and washed with water. It crystallised from alcohol in colourless needles m.p.151-52°. It showed no depression in melting point on admixture with the anilide obtained from 4-amino veratrol and phenyl acetic acid i.e. phenyl acet-3,6-dimethoxy anilide.

Anal.Calcd.for $C_{16}H_{17}O_3N$:	N, 5.17
Found:	N, 5.27

Selenium dioxide oxidation:

3:4-dimethoxyphenyl-benzyl ketone (1 gm), selenium dioxide (.43 gm) were heated under reflux with acetic anhydride (11 cc). After six hours the reactant mixture was filtered and poured into water, when it separated into a yellow solid on several crystallisations from benzene-petrol it gave light yellow needles of the diketone m.p.114.5-115°.

Anal.Calcd.for $C_{16}H_{14}O_4$:	C, 71.10; H, 5.22
Found:	C, 71.39; H, 5.31

2:3-Dimethoxydeoxybenzoin (Grignard method):

Benzyl magnesium chloride, obtained from benzyl chloride (11.18 gms) and magnesium turnings (2.3 gms), in the ethereal solution, as described earlier, was treated with finely powdered dry o-veratramide (4.0 gms) in small portions at a time. The rate of the addition of amide was determined by the vigour of the reaction. The solution was refluxed in an atmosphere of nitrogen, with occasional stirring for 48 hours. It was then hydrolysed with sulphuric acid (10 gms) at 0° and worked in the usual way when a brown viscous oil was obtained. The oil on fractionation gave (i) a colourless fragrant oil b.p.126-28°, 3 mm (0.91 g; 12% yield) and (ii) a thick yellow oil b.p.170-73°, a 3 mm (4.3; 76% yield).

The first fraction solidified to colourless shining plates m.p.54°. It did not show any depression in melting point when mixed with authentic sample of dibenzyl prepared by Clemmensen's method.

Anal.Calcd.fof $C_{14}H_{14}$: C, 92.30; H, 7.7

Found: C, 92.26; H, 7.65

The second fraction was redistilled when it gave a thick faint yellow oil b.p.170-73, 3 mm.

Anal.Calcd.for $C_{16}H_{16}O_3$: C, 74.97; H, 6.3

Found: C, 74.74; H, 6.1

It yielded 2:4-dinitrophenyl hydrazone m.p.180-82°, crystallised in reddish orange needles by ethyl acetate.

Anal.Calcd.for $C_{22}H_{20}N_4O_6$: N, 12.84

Found: N, 12.85

Semicarbazone: m.p.185-86°, (dilute alcohol)

Anal.Calcd.for $C_{17}H_{19}N_3O_3$: N, 13.40

Found: N, 13.41

Demethylation (2:3-dihydroxydeoxybenzoin):

The dimethoxydeoxybenzoin (1.3 gm) was refluxed with hydrobromic acid (Sp.Gr. 1.5; 30 cc) and glacial acetic acid (30 cc) for 5 hours. The mixture, on pouring into water separated into a solid. It was extracted with ether. The ether extract washed with water, then with sodium bicarbonate solution and again with water. It was dried over anhydrous magnesium sulphate. The ether was distilled off and the residual solid on repeated

crystallisations from petrol-benzene gave light yellow aggregates of shining needles m.p.79-81°.

Anal.Calcd.for $C_{14}H_{12}O_3$:	C, 73.66;	H, 5.3
Found:	C, 73.50;	H, 5.65

It yielded 2:4-dinitrophenylhydrazone m.p.281-82 (Ethyl acetate).

Anal.Calcd.for $C_{20}H_{16}N_4O_6$:	N, 13.71	
Found:	N, 14.00	

Selenium dioxide oxidation (2,3-dimethoxy benzil):

A mixture of 2:3-dimethoxydeoxybenzoin (1 gm.), selenium dioxide (0.43 gm.) and acetic anhydride (11.0 cc) was refluxed for 4 hours. On pouring the mixture into water a yellow thick oil was separated. It was extracted with ether and dried over anhydrous magnesium sulphate. On distilling off the ether a yellow oil was left behind, which distilled at 215-18°, 1.3 mm. The oil was crystallised from ethyl acetate-ligfoin mixture in yellow rhombic plates m.p.74°.

Anal.Calcd.for $C_{16}H_{14}O_4$:	C, 71.10;	H, 5.22
Found:	C, 71.36;	H, 5.31

2:4-dinitrophenylhydrazone: m.p.162-64° (ethyl acetate)

Anal.Calcd.for $C_{22}H_{18}N_4O_7$: N, 12.43

Found: N, 12.55

2:3-Dimethoxydeoxybenzoin (Organo zinc compound):

The Grignard reagent was prepared from 13.7 cc (0.13 moles) of freshly distilled benzyl chloride, 4.8 g (0.2 gram atoms) of magnesium and 60 cc of dry ether in an atmosphere of pure, dry nitrogen. The Grignard solution was added to a solution of 13.6 grams (0.1 mole) of anhydrous zinc chloride in dry ether (50 cc). The solution of Grignard reagent was added at such a rate that gentle refluxing was maintained. During the addition the mixture was vigorously stirred. After the addition of Grignard reagent, the mixture was heated at reflux temperature for about an hour and a half, during which time ether distilled till the volume of the mixture was reduced to about 60 cc. A solution of 14 gms. (0.7 mole) of o-veratroyl chloride in dry benzene (50 cc) (thiophene free) was added with stirring over fifteen minutes. The resulting mixture was well stirred and heated under reflux for additional 3 hours.

The reaction mixture was cooled in an ice bath and decomposed by careful addition of ice (60 gms) and water followed by sufficient amount of 20% sulphuric acid to give two phased. The aqueous phase was separated in a separating funnel and extracted with two 100 cc portions of benzene. The combine benzene extracts and the original benzene layer were placed in a separatory funnel, and washed successively with water (200 cc) and saturated sodium chloride solution (100 cc) and finally dried over sodium sulphate. After the recovery of the solvent the thick oil was left behind, which on distillation at 190-194°C gave a thick faint yellow oil (6.1 g, 34%) which yields 2:4-dinitrophenylhydrazone m.p.180-82°.

Anal.Calcd.for $C_{22}H_{20}N_4O_6$: N, 12.84

Found: N, 13.17

Alkali Degradation of 2:3-dihydroxydeoxybenzoin:

A mixture of 2:3-dihydroxydeoxybenzoin (0.4 g), potassium hydroxide (2.0 g.), water (2 cc), and methanol (3 cc) was heated to 240° and kept there for 40 minutes. After cooling, the mixture was diluted and acidified. The solution was extracted with ether. The ether extract was washed thoroughly with sodium bicarbonate and finally with water. The sodium bicarbonate solution on acidifica-

page 150 line 11.
Do you mean
diazotised p-nitraniline
or bis-diazotised
benzidine?

tion gave colourless precipitate which was filtered. It crystallised from boiling water in colourless plates m.p. 78°. No depression in melting point was observed when it was mixed with an authentic sample of phenyl acetic acid. The ether was recovered and the residue was taken in alcohol (.5 cc). It was subjected to chromatographic analysis on Whatman No.1 filter paper using butanol:acetic acid:water (30:10:20) as solvent mixture, employing ascending technique. The authentic sample of pyrocatechol was used as check. The spots were revealed by spraying with alcoholic ferric chloride and bis-diazotized p-nitroaniline solution. The two spots were found to be identical in Rf value and colour.

O-Hydroxydeoxybenzoin (Fries rearrangement):

It was prepared according to Venkataraman et al[253] by the Fries rearrangement of phenylphenyl acetate. The separation of the isomers formed was affected by distillation under reduced pressure and also by exhausting both the distillate and the residue with ligroin. The ligroin soluble fraction on concentration gave o-hydroxydeoxybenzoin in colourless hexagonal plates m.p.60° (24% yield). The residue on crystallisation from dilute alcohol was colourless crystals of p-hydroxydeoxybenzoin m.p.151°.

O-Methoxydeoxybenzoin (Grignard method):

O-Methoxyphenylbenzyl ketone was prepared by the reaction of Grignard reagent with O-methylbenzamide as detailed in earlier cases. A thick brown oil was obtained. The oil on fractionation gave a colourless fragrant liquid, which later solidified, in shining colourless plates m.p.54° (5% yield). It did not show any depression in melting point when it was mixed with the authentic sample of dibenzyl. The second fraction was collected as thick oil of light yellow colour b.p.198-200°, 9 mm (62% yield).

Anal.Calcd.for $C_{15}H_{12}O_2$: C, 80.36; H, 5.36

Found: C, 80.10; H, 5.21

It yielded 2:4-dinitrophenyl hydrazone: m.p.162-64°

Anal.Calcd.for $C_{21}H_{18}N_4O_5$: N, 13.793

Found: N, 13.503

Semicarbazone: m.p.214-16 (Lit.[235] m.p.212-14°)

Anal.Calcd.for $C_{16}H_{17}O_2N_3$: N, 14.84

Found: N, 14.651

O-Hydroxydeoxybenzoin:

O-methoxydeoxybenzoin (2.2 gms) was demethylated with hydrobromic acid (Sp.Gr. 1.5; 40 cc) and glacial acetic acid (40 cc) exactly in the manner as described earlier. The product on crystallisation from light petroleum melted at 60°. It showed no depression in melting point when mixed with the previously prepared sample of O-hydroxydeoxybenzoin.

Anal.Calcd.for $C_{14}H_{10}O_2$: C, 80.00; H, 4.76

Found: C, 79.63; H, 4.40

2:4-dinitrophenylhydrazone m.p.217-19° (Lit.[253] m.p.219°)

Anal.Calcd.for $C_{20}H_{16}N_4O_5$: N, 14.285

Found: N, 13.971

Semicarbazone m.p.200

Anal.Calcd.for $C_{15}H_{15}O_2N_3$: N, 15.687

Found: N, 15.291

Oxime m.p.124°

Anal.Calcd.for $C_{14}H_{13}NO_2$: N, 6.123

Found: N, 6.446

Interaction of Benzyl magnesium chloride and o-Nitrobenzamide:

o-Nitrobenzamide was treated with Grignard reagent as in the previous cases. The Grignard compbex on working up in the usual manner yielded a deep yellow oil, which on distillation under reduced pressure gave a light yellow product. This on repeated crystallisations from methyl alcohol gave a colourless fragrant product m.p.54°. It showed no depression in melting point when mixed with an authentic sample of dibenzyl.

Dibenzyl:

Benzil (5.0 gms) on reduction according to Clemmensen gave a colourless fragrant product which was filtered. The dry product on crystallisation from methanol gave colourless fragrant crystals of dibenzyl (4.5 g) m.p.54°.

8-Hydroxy Isoflavone:

(a) Ethyl formate-sodium method:

A solution of 2:3-dihydroxydeoxybenzoin (0.5 g) in freshly distilled ethylformate (15 cc), cooled to 0°, was gradually added with stirring to pulverised sodium (0.5 g). The mixture was left in an ice chest for 48 hours. Pieces of ice and hydrochloric acid (15 cc) were then added and the mixture stirred well and left over night. Unreacted

ethyl formate was then distilled off under reduced pressure when a dark brown solid was separated, which was filtered off and washed with water. On repeated crystallisations from dilute ethyl alcohol it gave colourless shining needles (0.25 g) m.p.222-24°. When subjected to sodium amalgam test it gave positive test but no colour with alcoholic ferric chloride. It dissolved in aqueous sodium carbonate and sodium hydroxide giving a yellow solution.

Anal.Calcd.for $C_{15}H_{10}O_3$: C, 75.59 ; H, 4.23

Found: C, 75.38 ; H, 4.27

Acetylation:

The isoflavone (0.2 gm) and fused sodium acetate (0.3 gm) were refluxed with acetic anhydride for one hour on a sand bath. The solid obtained on pouring the mixture over crushed ice was washed thoroughly with water and dried. It was crystallised from methyl alcohol in colourless needles m.p.160°.

Anal.Calcd.for $C_{17}H_{12}O_4$: C, 72.84; H, 4.31

Found: C, 72.98; H, 4.298

Methylation:

A solution of the isoflavone (0.4 gm) in dry acetone (100 cc) was treated under reflux with dimethyl sulphate

(2.5 cc) and anhydrous potassium carbonate (8 gms) for 30 hours. It was filtered and the residue washed with hot acetone. On distilling off the solvent a straw coloured solid was obtained which was crystallised from methyl alcohol (charcoal) into shining colourless needles m.p.163°.

Anal.Calcd.for $C_{16}H_{12}O_3$:	C, 76.19;	H, 4.76
Found:	C, 76.31;	H, 4.85

Ethoxalylation; 2-Carbethoxy-8-hydroxy isoflavone:

Redistilled ethoxalyl chloride (1.8 g) was slowly added with strong stirring into an ice cold solution of 2:3-dihydroxydeoxybenzoin (1 g) in pyridin (10 cc). After the addition the mixture was left for 24 hours at room temperature. The mixture was poured into water and extracted with chloroform. The chloroform extract was washed successively with 10% hydrochloric acid and water and dried over magnesium sulphate. The chloroform was distilled off. The product left was crystallised from ethanol in colourless crystals m.p.213-15°.

Anal.Calcd.for $C_{18}H_{14}O_3$:	C, 69.64;	H, 4.54
Found:	C, 69.74;	H, 4.37

2-Carboxy-8-hydroxy Isoflavone:

The above ester (0.6 g) in ethanol was warmed for four hours with excess of 5% aqueous sodium carbonate. The organic solvent was evaporated and the cold solution was acidified when it gave a colourless solid. The solid was filtered, washed with water and dried. On crystallisation from dilute methanol it gave shining needles m.p.262-63°.

Anal.Calcd.for $C_{16}H_{10}O_5$: C, 68.07; H, 3.57
Found: C, 67.90; H, 3.687

8-Hydroxy Isoflavone:

The above carboxy isoflavone was decarboxylated by heating rapidly in portions (ca.50 mg) to 275° till the evolution of carbondioxide ceased. The crude melt was extracted with ether, washed with aqueous sodium bicarbonate and then with water. It was dried over anhydrous magnesium sulphate. The ether was recovered, and the residue on crystallisation from dibute alcohol (charcoal), gave colourless shining needles m.p.222-24°. The melting point was found undepressed on admixture with the sample obtained by ethylformate sodium synthesis.

Anal.Calcd.for $C_{15}H_{10}O_3$: C, 75.59; H, 4.23
Found: C, 75.40; H, 4.23

Alkaline hydrolysis:

The isoflavone (0.3 g) in methanol (7 cc) and water (5 cc) containing sodium hydroxide (1 g) was refluxed for 12 hours. The alcohol was evaporated and the mixture was diluted with water and acidified. It was extracted with ether, washed with water, then with sodium bicarbonate solution and finally with water. Ether was recovered, the residual solid was taken into small quantity of alcohol, and was subjected to chromatographic analysis on a Whatman No.1 filter paper using butanol:acetic acid: water (60:10:20) as solvent mixture, employing the ascending technique. The chromatogram was run alongside with an authentic sample of 2:3-dihydroxydeoxybenzoin. The spots were revealed by spraying with alcoholic ferric chloride and diazotized p-nitroaniline solution and were found to be identical.

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P U B L I C A T I O N S

(i) Kaempferol from Flowers of Ervatamia Coronaria Stapf.

Naturwissenschaften,1959,46,401-2.
(reprint attached)

(ii) Synthesen von 3,4-Dihydroxy-deoxybenzoin mittels Bortrifluorids bzw. der Grignard-Methode. Konfiguration des Oxims.

Ber., 1959,92,2555-2559 (reprint attached)

(iii) Grignard-Reaktionen hei der Synthese Von Desoxybenzoin, II. Synthese Von 8-Hydroxy-isoflavone.

Ber., 1961. (in the press).

(iv) Flavonoids ds flower d'Argemone Mexicana Linn.

Compt.rend.,1961,252,1974.

(v) Flavonoids from Argemone Mexicana Linn. (papaveraceae)

J.Org.Chem., 1961. (in the press).

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Kaempferol from Flowers of Ervatamia Coronaria Stapf

On account of its immense therapeutic value[1]) Ervatamia Stapf., Syn., Tabernaemontana R. Br. (Apocynaceae) early attracted attention. Although the milky latex, the bark and the root of the genus have been extensively examined for various plant products, no mention so far appears to have been made of the presence of flavonoids. The isolation of a crystalline flavonoid compound from the flowers of the species is now reported.

The ethanol extract on purification by suitable solvent fractionation, and subsequent treatment with neutral and basic lead acetate yielded a semi-solid product. The cyanidin test[2]) indicated the presence of flavonoids, and chromatographic examination revealed the presence of a single flavonoid compound. A yellow solid was obtained by hydrolysis of the semi-solid which was purified by fractionation on alumina, and yielded light yellow needles after several crystallizations from dilute pyridine. The acidic cyanidin and WILSON's boric acid tests[2]),[4]) were positive confirming the presence of a flavonol. It was identified as Kaempferol by its melting point 276 to 278° [lit.[3]) 276 to 278°] and mixed melting point with an authentic sample of Kaempferol and gave an acetate, m.p. 180 to 182° [lit.[3]) m.p. 181°].

Further confirmation of the identity of the aglycone was furnished by co-chromotography [cf. [5]),[6])]. The spots were revealed in U.V. light, U.V. light and ammonia vapours, by spraying with solutions of ferric chloride, diazotized p-nitro aniline and bis-diazotized benzidine, R_F 0·86. The aglycone on microdegradation[6]),[7]) followed by the chromatographic examination of the fragments revealed two spots by spraying with diazotized p-nitro-aniline and sodium carbonate, and bis-diazotized benzidine, indistinguishable from those of authentic samples of phloroglucinol and p-hydroxy benzoic acid, R_F (phenol) 0·69; R_F (acid) 0·87.

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Dept. of Chemistry, Muslim University, Aligarh (India)
M.O. FAROOQ, W. RAHMAN and M. ILYAS

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MOHAMMAD OMAR FAROOQ, WASIUR RAHMAN und
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Synthesen von 3.4-Dihydroxy-desoxybenzoin mittels Bortrifluorids bzw. der Grignard-Methode. Konfiguration des Oxims

Department of Chemistry, Muslim University, Aligarh, Indien

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(Eingegangen am 28. April 1959)

3.4-Dihydroxy-desoxybenzoin und sein Dimethyläther wurden mit Hilfe von Bortrifluorid in beträchtlich erhöhten Ausbeuten synthetisiert, der Dimethyläther in noch höherer Ausbeute mittels der Grignard-Methode. 3.4-Dimethoxybenzil, Phenylessigsäure-[3.4-dimethoxy-anilid] und *N*-Benzyl-[3.4-dimethoxybenzamid] werden erstmals beschrieben. Durch Beckmann-Umlagerung wird gezeigt, daß das Ketoxim des 3.4-Dimethoxy-desoxybenzoins die Konfiguration des *syn*-Benzyltyps besitzt.

Substituierte Desoxybenzoine zogen in den vergangenen Jahren wegen ihrer medizinischen Bedeutung[1] und wegen ihrer Verwendbarkeit als Zwischenprodukte zur Synthese von Stilböstrol-Analoga[2] die Aufmerksamkeit auf sich. 3.4-Dihydroxy-desoxybenzoin und sein Dimethyläther I wurden früher durch Nencki's und Friedel-

[1] J. R. GEIGY AG, Engl. Pat. 728280; C. A. **50**, 5756 [1956].
[2] T. C. MYERS, R. J. PRATT, R. L. MORGAN, J. O'DONELL und E. V. JENSEN, J. Amer. chem. Soc. **77**, 5655 [1955].

Crafts'-Reaktionen[3-5] bereitet, mit Ausnahme eines Falles (23% d. Th.[4]) wurden jedoch keine Ausbeuteangaben gemacht. Im Verlauf unserer Arbeit über die Synthese von Isoflavonen gelang uns die im folgenden beschriebene Verbesserung der Ausbeute von 3.4-Dihydroxy-desoxybenzoin. Ein weiterer Gesichtspunkt für diese Arbeit ergab sich aus der Tatsache, daß die Konfiguration des Ketoxims von 3.4-Dimethoxy-desoxybenzoin nicht bekannt war.

Leitete man Bortrifluorid in ein Gemisch von Brenzcatechin und Phenylessigsäure in Chloroform ein, so entstand in Ausbeuten um 36% d. Th. 3.4-Dihydroxy-desoxybenzoin. Ersetzte man Brenzcatechin durch Veratrol, so erhielt man die Dimethoxyverbindung I in 65-proz. Ausbeute.

C. BEIS' Methode[6] zur Herstellung aliphatischer Ketone mit den durch S. S. JENKINS[7] erzielten Verbesserungen wurden ebenso erfolgreich auf die Synthese von I angewandt. Veratrumsäure-amid[8] (1 Mol.) setzte man allmählich einem Überschuß (3—4 Moll.) von Benzylmagnesiumchlorid in Äther zu und erhitzte bei gelegentlichem Umrühren auf dem Wasserbad 48 Stdn. unter Rückfluß. Der so erhaltene Grignard-Komplex lieferte, in üblicher Weise aufgearbeitet, reines kristallisiertes I in 71-proz. Ausbeute. Keinerlei Anzeichen für irgendein anomales Produkt[9] wurde hierbei beobachtet. Der Dimethyläther I ließ sich mit Bromwasserstoffsäure in Eisessig in 70-proz. Ausbeute entmethylieren.

Die Identität des Dihydroxy-desoxybenzoins wurde bestätigt durch Schmelzpunkt und Misch-Schmelzpunkt mit einer frisch nach der Methode von FINZI[3] und LESPAGNOL und Mitarbb.[4] hergestellten Probe sowie durch Darstellung seines 2.4-Dinitro-phenylhydrazons. 3.4-Dimethoxy-desoxybenzoin (I) gab bei der Entmethylierung ein Produkt, welches im Gemisch mit 3.4-Dihydroxy-desoxybenzoin keine Schmelzpunktsdepression zeigte. Die Identität von I wurde weiterhin erhärtet durch Bereitung des Oxims (Schmp. 128—129°) und des Phenylhydrazons (Schmp. 137 bis 138°). Der Schmelzpunkt des letzteren stieg auch bei wiederholten Versuchen nicht höher (A. J. CHALMERS und F. LIONS[10] fanden 153—154°). I ergab auch leicht ein 2.4-Dinitro-phenylhydrazon und ein Semicarbazon. Mit Selendioxyd in Acetanhydrid oxydiert, lieferte I ein Produkt, dessen Analysendaten gut auf das Diketon zutrafen und das ein 2.4-Dinitro-phenylhydrazon bildete.

Wir ergänzten die Charakterisierung von I ferner noch durch eine Studie über die Beckmann-Umlagerung seines Ketoxims. I wurde oximiert und ergab unter unseren Versuchsbedingungen nur *ein* Oxim, dem entweder die Formel IIa oder IIb zuzuordnen ist. Um die Konfigutarion dieses Oxims zu ermitteln, behandelten wir es mit Phosphorpentachlorid in Äther, wobei eines der Anilide IIIa oder IIIb entstand. Da diese beiden Anilide unbekannt waren, haben wir sie aus 4-Amino-veratrol[8,11] und

[3] F. FINZI, Mh. Chem. **26**, 1119 [1905]; J. C. S. Abstr. I **1905**, 907.
[4] A. LESPAGNOL, J. TURLUR und L. LESPAGNOL, Bull. Sci. Pharmacol. **46**, 305 [1939]; C. A. **33**, 8182 [1939].
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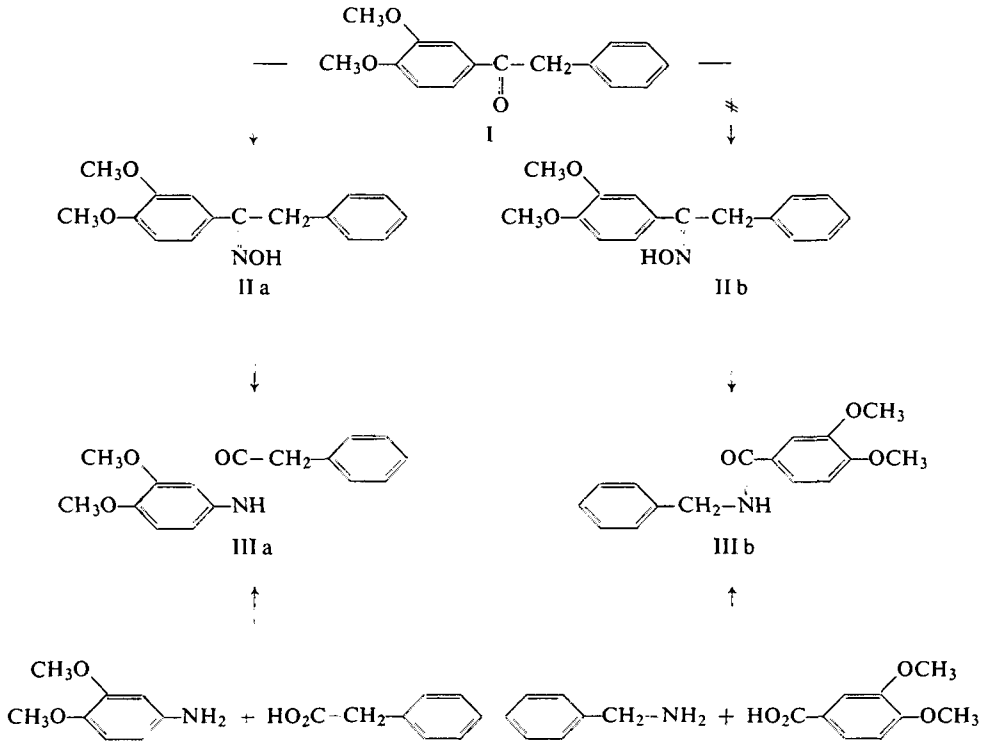
[8] H. MEYER, Mh. Chem. **22**, 429 [1901]; J. C. S. Abstr. **1901**, 628.
[9] P. R. AUSTIN und J. R. JOHNSON, J. Amer. chem. Soc. **54**, 647 [1932].
[10] A. J. CHALMERS und F. LIONS, J. Proc. Roy. Soc. New South Wales **67**, 178 [1933]; C. A. **1934**, 765.
[11] J. S. BUCK und W. S. IDE, Org. Syntheses **16**, 4 [1936].

Phenylessigsäure im einen Fall bzw. aus Benzylamin[12] und Veratrumsäure im andern Fall synthetisiert. Vergleich des durch Beckmann-Umlagerung gewonnenen Produktes erwies dessen Identität mit Phenylessigsäure-[3.4-dimethoxy-anilid] (IIIa) und legte damit die Konfiguration des Oxims als zum *syn*-Benzyltyp gehörig fest. Das folgende Schema verdeutlicht die verschiedenen Reaktionsschritte:



BESCHREIBUNG DER VERSUCHE[13,14]

3.4-Dihydroxy-desoxybenzoin (Bortrifluorid-Methode): Man löste 35 g *Phenylessigsäure* in 60 g Chloroform und ließ durch die auf 10° gekühlte Lösung einen Strom von Bortrifluorid perlen. Setzte man 15 g *Brenzcatechin* zu, so zerfiel der Phenylessigsäure-Bortrifluorid-Komplex nach einiger Zeit, und man setzte das Einleiten von Bortrifluorid eine weitere Viertelstunde fort. Man beließ die Reaktionsmischung über Nacht, schüttete auf zerkleinertes Eis und ließ 2 Stdn. stehen. Nun extrahierte man mit Äther, trennte die Äther/Chloroform-Schicht ab, wusch mit Natriumhydrogencarbonatlösung und mit Wasser und trocknete über Natriumsulfat. Der Verdampfungsrückstand, ein bräunlichgelbes Öl, erstarrte später und lieferte, aus Wasser, anschließend aus Äthanol/Benzol umkristallisiert, nahezu

[12] H. R. ING und R. H. F. MANSKE., J. chem. Soc. [London] **1926**, 2348.
[13] Alle Schmpp. sind unkorrigiert.
[14] Die Mikroanalysen wurden vom Chemistry Department der M. S. University of Baroda ausgeführt.

farblose Nadeln vom Schmp. 173–174° (Lit.[3]: Schmp. 173°). Ausb. 11.2 g (36% d. Th.; Lit.[4,7]: 23% d. Th.).

$C_{14}H_{12}O_3$ (228.2) Ber. C 73.66 H 5.29 Gef. C 73.58 H 5.05

Die Verbindung war im Gemisch mit einer nach FINZI[3] (Nencki's Methode) bereiteten Probe ohne Schmelzpunktsdepression.

2.4-Dinitro-phenylhydrazon: Schmp. 243° (aus Essigester).

$C_{20}H_{16}N_4O_6$ (408.4) Ber. N 13.71 Gef. N 13.60

3.4-Dimethoxy-desoxybenzoin (I)

a) *Nach der Bortrifluorid-Methode:* Aus *Veratrol* an Stelle von Brenzcatechin erhielt man, wie oben beschrieben, 65% d. Th. *I* als bräunliches Öl, das, aus Benzol/Petroläther umkristallisiert, farblose Aggregate seidiger Nadeln vom Schmp. 87–88° lieferte (Lit.: Schmp. 88°[5]; 91–93°[15]).

$C_{16}H_{16}O_3$ (256.3) Ber. C 74.97 H 6.29 Gef. C 74.75 H 6.06

Oxim: Schmp. 128–129° (Lit.[15]: Schmp. 128–129°).

$C_{16}H_{17}NO_3$ (271.3) Ber. N 5.16 Gef. N 5.15

Phenylhydrazon: Schmp. 137–138° (Lit.[10]: Schmp. 153–154°).

$C_{22}H_{22}N_2O_2$ (364.4) Ber. N 8.09 Gef. N 8.09

Semicarbazon: Schmp. 189–190°.

$C_{17}H_{19}N_3O_3$ (313.3) Ber. N 13.40 Gef. N 13.17

2.4-Dinitro-phenylhydrazon: Schmp. 198–199°.

$C_{22}H_{20}N_4O_6$ (436.4) Ber. N 12.84 Gef. N 13.27

b) *Nach der Grignard-Methode: Benzylmagnesiumchlorid,* aus 11.4 g Benzylchlorid und 4 g Magnesiumspänen auf übliche Weise in Ätherlösung bereitet, wurde portionsweise mit 3 g fein gepulvertem, trockenem *Veratrumsäure-amid*[8] behandelt. Die Zugabegeschwindigkeit des Amids wählte man nach der Heftigkeit der Reaktion. Man erhitzte die Lösung unter gelegentlichem Rühren 48 Stdn. in Wasserstoffatmosphäre unter Rückfluß, hydrolysierte sodann bei 0° mit 10-proz. Schwefelsäure und arbeitete das ölige Reaktionsprodukt in üblicher Weise auf. Das Öl lieferte, aus Petroläther/Benzol umkristallisiert, 4 g (71% d. Th.) feine seidige Nadeln vom Schmp. 87–88°.

Ätherspaltung: 6.5 g *I* wurden mit 150 ccm Bromwasserstoffsäure (*d* 1.5) und 150 ccm Eisessig 5 Stdn. unter Rückfluß erhitzt. Man goß die Mischung anschließend in Wasser, extrahierte mit Äther und erhielt als Verdampfungsrückstand ein bräunliches Öl, das aus Wasser kristallisierte. Schmp. 173–174°.

Selendioxyd-Oxydation von *I:* 1 g I erhitzte man mit 0.43 g Selendioxyd in 11 ccm Acetanhydrid 4 Stdn. unter Rückfluß und goß in Wasser, sobald sich ein gelber Niederschlag abschied. Mehrfaches Umkristallisieren des letzteren aus Petroläther/Benzol lieferte das *Diketon* in hellgelben Nadeln vom Schmp. 114.5–115°.

$C_{16}H_{14}O_4$ (270.3) Ber. C 71.10 H 5.22 Gef. C 71.39 H 5.31

Das *2.4-Dinitro-phenylhydrazon* wurde leicht erhalten. Schmp. 223–224°.

$C_{22}H_{18}N_4O_7$ (450.4) Ber. N 12.43 Gef. N 12.31

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[15] M. TIFFENEAU, A. ORYEKHOV und M. ROGER, Bull. Soc. chim. France **49**, 1757 [1931], C. A. **1932**, 2424.

Oxim von I: Man vermischte 5 g Hydroxylamin-hydrochlorid, 5 ccm Wasser, 20 ccm 10-proz. Natronlauge und 2 g I und setzte gerade bis zur klaren Lösung Äthanol zu. Nun erhitzte man 15 Min. unter Rückfluß und beließ über Nacht im Eisschrank. Das abgeschiedene ölige Produkt ergab, mehrmals aus verd. Äthanol umkristallisiert, das Oxim in glänzenden Nadeln vom Schmp. 128–129° (Lit.[10]: Schmp. 128–129°).

$C_{16}H_{17}NO_3$ (271.3) Ber. N 5.16 Gef. N 5.15

N-Benzyl-[3.4-dimethoxy-benzamid] (IIIb): 1 g *Benzylamin* erhitzte man mit 1 g *Veratrumsäure* 3 Stdn. auf 180–190°, zerrieb das Reaktionsprodukt zuerst mit verd. Natronlauge, dann mit verd. Salzsäure und wusch schließlich mit Wasser. Aus Benzol/Petroläther umkristallisiert, lieferte die rohe Masse farblose Nadeln vom Schmp. 134–136°.

$C_{16}H_{17}NO_3$ (271.3) Ber. N 5.17 Gef. N 5.21

4-Amino-veratrol (3.4-Dimethoxy-anilin): 5.8 g *Veratrumsäure-amid*, aus Veratroylchlorid und konz. Ammoniak[9] bereitet, lieferte mit alkalischem Natriumhypochlorit[11] in üblicher Weise 3.9 g *4-Amino-veratrol* vom Schmp. 87–88°.

Phenylessigsäure-[3.4-dimethoxy-anilid] (IIIa): Aus 1 g *4-Amino-veratrol* und 1 g *Phenylessigsäure* gewann man das Anilid, wie bei IIIb beschrieben. Farblose Nadeln vom Schmp. 151–152° (aus Äthanol).

$C_{16}H_{17}NO_3$ (271.3) Ber. N 5.17 Gef. N 5.27

Im Gemisch mit dem durch Beckmann-Umlagerung des Oxims IIa erhaltenen Produkt schmolz das Anilid ohne Depression.